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Food and Drug Administration

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CHEMICAL COMPOUNDS IN FOOD-PRODUCING ANIMALS

Criteria and Procedures for Evaluating Assays for Carcinogenic Residues

[4110-03-M]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Parts 70, 500, 514, 571]

[Docket No. 77N-0026]

CHEMICAL COMPOUNDS IN FOOD-PRODUCING ANIMALS

Criteria and Procedures for Evaluating Assays for Carcinogenic Residues

AGENCY: Food and Drug Administration.

ACTION: Proposal.

SUMMARY: The Food and Drug Administration (FDA) is proposing to establish procedures and minimum criteria to ensure the absence of cancer-causing residues in edible products of food-producing animals to which drugs, food additives, or color additives have been administered. This is a reproposal of regulations revoked in accordance with a court order.

DATES: Comments by July 18, 1979. Notices of participation for the public hearing by May 4, 1979. Public hearing before the Commissioner June 4, 1979.

ADDRESSES: Comments and notices of participation are to be submitted to the Hearing Clerk (HFA-305), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857.

FOR INFORMATION ON THIS PROPOSAL, CONTACT:

Robert J. Condon, Bureau of Veterinary Medicine (HFV-105), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20857, 301-443-1580.

FOR FURTHER INFORMATION ON THE HEARING BEFORE THE COMMISSIONER CONTACT:

Constantine Zervos, Director, Scientific Liaison and Intelligence Staff (HFY-31), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20857, 301-443-4490.

SUPPLEMENTARY INFORMATION: These proposed regulations would provide an operational definition of the no-residue requirement of the so-called "DES proviso" to the anticancer clauses, sections 409(c)(3)(A), 512(d)(1)(H), and 706(b)(5)(B), of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 348(c)(3)(A), 360(d)(1)(H), and 376(b)(5)(B)). The regulations also propose to establish criteria for accepting assays and procedures for establishing suitable postadministration withdrawal periods to prevent the occurrence of carcinogenic residues in edible animal products.

Prior to July 19, 1973, FDA had applied the DES proviso on a case-by-case basis, without published criteria. However, the Commissioner of Food and Drugs concluded that it was appropriate to establish criteria and procedures for their application through rulemaking to permit public discussion of the scientific, legal, and policy issues involved. Accordingly, the Commissioner proposed a set of regulations, in the FEDERAL REGISTER of July 19, 1973 (38 FR 19226), and afforded 60 days for public comment.

The numerous comments received were submitted by scientists affiliated with consumer groups, universities, scientific societies, State and Federal agencies, trade associations, and affected manufacturers; some were from nonaffiliated individuals. Many comments revealed a sharp divergence of opinion concerning FDA's interpretation of the proviso to the aniticancer clauses of the act.

The Commissioner promulgated the final regulations in the FEDERAL REGISTER of February 22, 1977 (42 FR 10412), but solicited comments on four specific issues: (1) The acceptable level of risk, (2) comparative metabolism, (3) regulation of endogenous compounds, and (4) methods of determining an assay's lowest limit of reliable measureent. On March 23 and 24, 1977, the Animal Health Institute (AHI) and three other groups petitioned the Commissioner to stay the effective date of the regulations and to then revoke them. The Commissioner denied these petitions on April 27. In response to a separate request by AHI, however, the Commissioner extended the comment period to July 25, 1977 (42 FR 24254).

On May 12, AHI filed a complaint in the United States District Court for the District of Columbia alleging that the regulations were unlawful: (1) because they broadened the scope of the Delaney, i.e., anticancer, clause of the act to include substance that have not been determined to be carcinogenic, and (2) because they foreclosed marketing of a compound unless there exists an assay of sufficient "sensetivity" to detect residues of the compound at "theoretically" safe levels determined by the regulations. Also, AHI alleged that the regulations were impractical and embodied novel on highly suspect technical principles that would impose enormous financial and environmental costs on the animal health industry. Finally, it alleged that the final regulations violated the Administrative Procedure Act (5 U.S.C. 551 note) because they departed from and radically changed the proposed regulations and were not republished for comment.

Based on AHI's affidavits contending that the statistical procedure for extrapolation of animal data adopted in the final order was significantly different from and more complex than that proposed, and perhaps improperly interpreted, the court remanded the case to the agency for further consideration. The court also required the agency to assess the question raised by AHI about the technical feasibility of the regulations, and it suggested that the Commissioner repropose the regulations (*Animal Health Institute* v. *Food and Drug Administration*, Civil No. 77—806 (D.D.C. Feb, 8, 1978)). In accordance with the court's order, the Commissioner revoked the regulations on May 26, 1978 (43 FR 22675) and is now reproposing all the regulations for public comment. In this proposal, the Commissioner has evaluated and responded to AHI's allegations, the court's questions, the citizen petitions to revoke the regulations, and all comments filed on the final order. (For the sake of clarity, the final order is hereafter designated the "February notice" or the "1977 notice".)

Since the July 1973 proposal, the Commissioner has used the risk assessment element of the regulations as the prototype for segments of the agency's anticancer policy. Before attempting to build a uniform procedure for regulating all chemicals in the food supply, the Commissioner has adopted where appropriate, the best elements of the emerging scientific and regulatory procedures of risk assessment, metabolism studies, in vitro mutagenesis tests, etc., for regulating residues in food derived from food-producing animals.

The Commissioner selected this class of compounds as the test model because FDA has premarket approval authority over the chemicals intentionally used in these animals, and the DES proviso to the Delaney clause has made regulation of these compounds one of the agency's most difficult tasks.

Based on experience with the principles outlined in the proposal, gained through several years of regulating these chemicals on a case-by-case basis, the Commissioner believes that they have potential applicability for regulating all compounds covered by the act. Moreover, due to the extensive interest in the issues, the Commissioner now believes that the time is ripe for formulating a comprehensive approach for regulating all chemical carcinogens. Expanding the use of the principles set out in these regulations into other areas regulated by the agency seems desirable from the perspectives of science and public health protection, but the results of their ex-

panded use, e.g., cost, cannot now be calculated.

Because an error in selecting the basic principles could lead to a future tragedy, the principles adopted at this time must be reasonable and must not underestimate the potential risks associated with the use of chemicals. Accordingly, the Commissioner is proposing to adopt principles that some may consider too "conservative." The term "conservative," however, is relative. Further, although the principles form an integrated scheme of regulation, individual segments can be severed and replaced.

For all the foregoing reasons, the Commissioner has determined that, in addition to the 120-day comment period for filing written comments, an informal public hearing should be held in accordance with Part 15 (21 CFR Part 15). The informal public hearing will provide an open forum for the presentation of information, views, and discussions on all aspects of the proposal. Because the general principles articulated in the regulations have widespread potential use, the Commissioner asks that the witnesses focus on the principles that form the basis of the regulations, in addition to the issue of the technical feasibility of the required analytical technology. In particular, the Commissioner requests discussion of the following:

1. Threshold assessment procedures.
2. Criteria for selecting residues for chronic toxicity testing.
3. The types of investigations necessary to study how chemicals are metabolized, and the role of these studies in assessing the parent compound's safety.
4. The use of comparative metabolism studies for selecting the laboratory animal species to be used as surrogates for man in chronic toxicity testing.
5. The utility of short-term in vitro mutagenesis tests in assessing the safety of a compound.
6. Mathematical risk estimation procedures, including (a) methods of assessing risks within a species and (b) methods of cross-species extrapolation.
7. Procedures for combining data from the same or different carcinogenesis bioassays.
8. The regulation of endogenous substances.
9. The acceptable level of risk.

In preparing final regulations, the Commissioner will consider the administrative record of this hearing along with all other written comments received during the comment period specified in this proposal and on the transcript of the Part 15 hearing.

The hearing will be held on June 4, 1979, starting at 9 a.m. in the Washington, DC area at a place to be announced later.

A written notice of participation must be filed in accordance with § 12.45 (21 CFR 12.45) with the Hearing Clerk (HFA-305), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857, not later than May 4, 1979. The envelope containing the notice of participation, and the notice of participation itself, should be prominently marked "SOM Hearing." The notice of participation must also contain Hearing Clerk Docket No. 77N-0026, the name, address, and telephone number of the person desiring to make a statement, along with any business affiliation, the text of the presentation, and the approximate length of time requested for the presentation. The Commissioner is requiring submission of the text of all presentations before the hearing to promote a comprehensive discussion of the issues, but the Commissioner recognizes that some revisions in the text before the hearing may be necessary. A schedule for the hearing will be mailed to each person who files a notice of participation; the schedule will also be available from the FDA Hearing Clerk. Individuals and organizations with common interests are urged to consolidate or coordinate their presentations.

If the responses to this notice of hearing are so numerous that insufficient time is available to accommodate the full amount of time requested in the notices of participation received, the Commissioner will allocate the available time among the persons making the oral presentation to be used as they wish. Final versions of written statements (preferably four copies) should be presented to the presiding officer on the day of the hearing or submitted to the Hearing Clerk by June 19, 1979 for inclusion in the administrative record.

The plenary hearing will be open to the public, and any interested person who has filed a written notice of participation may be heard concerning matters raised in the written statement which are relevant to the issues under consideration.

Additional comments from interested persons may be submitted during the period following the hearing until the end of the comment period.

I. Introduction

A. Statutory Background

1. Food Additives Amendment of 1958

Section 409 of the Federal Food, Drug, and Cosmetic Act (Food Additives Amendment of 1958, Pub. L. 85-929) establishes criteria and prescribes procedures for FDA's premarket review and approval of food additives that have been shown to be safe. Section 409 was enacted to protect consumers by requiring substances that are intentionally added to food, or may reasonably be expected to become components or otherwise affect the characteristics of food, to be shown to be safe through rigorous scientific testing procedures. As the legislative history of the amendment demonstrates, one primary function was to protect the health of consumers by requiring manufacturers of food additives and food processors to test any potentially unsafe substances that are added to food in accordance with principles deemed appropriate by qualified scientists (Ref. 1).

Before the amendment, FDA's authority for ensuring the safety of food additives was limited to sections 402(a)(1) and 402(a)(2)(A) as enacted in 1938. Under these sections the agency must show that an intentionally added food substance may be injurious to health. Thus, the agency has to test the poisonous or deleterious substance before taking action. Therefore, the amendment shifted the burden of both testing and proving safety to the proponent of the additive.

When the Committee on Interstate and Foreign Commerce reported the bill to the full House of Representatives, the bill did not contain an anticancer clause, but it did contain a section requiring the premarketing testing of food additives to demonstrate safety. That section is now known as the general safety provision (section 409(c)(3)(A)). After the bill was reported out, Congressman Delaney suggested the addition of the anticancer proviso to the bill, and the following proviso was added to the bill as a Committee amendment on August 13, 1958:

> • • • *Provided*, That no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal • • •.

Reportedly to assure enactment of the legislation, the Committee and the Department of Health, Education, and Welfare (HEW) agreed to the amendment, but in a letter to the Chairman of the Committee, then Assistant Secretary Elliot L. Richardson noted that the amendment did not change the meaning of the bill. Moreover, the letter also illustrates the interaction between the general safety and anticancer provisions of the bill and the broad scope that the Delaney anticancer clause is to be given. It makes clear that the anticancer clause is a corollary of the general safety clause; and that compounds, even when subject to the anticancer clause, are also subject to the general safety clause:

> This Department is in complete accord with the intent of these suggestions—that

no substance should be sanctioned for use in food that might produce cancer in man. H.R. 13254, as approved by your committee, will accomplish this intent, since it specifically instructs the Secretary not to issue a regulation permitting the use of an additive in food if a fair evaluation of the data before the Secretary fails to establish that the proposed use of the additive will be safe. The scientific tests that are adequate to establish the safety of an additive will give information about the tendency of an additive to produce cancer when it is present in food. Any indication that the additive may thus be carcinogenic would, under the terms of the bill, restrain the Secretary from approving the proposed use of the additive unless and until further testing shows to the point of reasonable certainty that the additive would not produce cancer and thus would be safe under the proposed conditions of use. This would afford good, strong public health protection (Ref. 2).

As enacted in 1958, the anticancer (or so-called Delaney) clause of section 409 flatly proscribed the approval of any additive if after "a fair evaluation of the data before the Secretary" the additive "is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal * * *." As applied to additives added directly to human food, this language has remained unchanged, although hotly debated. Accordingly, as a legal matter, section 409 precludes a finding by FDA that a direct food additive that has been shown, by ingestion or other appropriate studies, to cause cancer in laboratory animals (or, of course, in man) can be safely added to food, in any amount, for any purpose.

2. Color Additive Amendments of 1960

The Color Additive Amendments of 1960 (Pub. L. 86-618) added a provision to the basic act for colors that is directly analogous to the food additives provision. Petitioners for color additive regulations must demonstrate by rigorous testing the safety of these additives before they can be approved by FDA for addition to food, drugs, or cosmetics. In addition, the amendments added another anticancer clause to the act.

The legislative history of the Color Additive Amendments of 1960 describes the congressional and executive (HEW) concern about the potential carcinogenicity of these color additives; nevertheless, the Secretary of HEW again explained that an express anticancer clause was unnecessary to prevent approval of carcinogenic or potentially carcinogenic color additives because it did not provide any public protection that is not already provided by the general safety clause (Ref. 3).

3. Drug Amendments of 1962

In 1962, Congress culminated several years of hearings on the drug industry by enacting the Drug Amendments of 1962 (Pub. L. 87-781); the infamous thalidomide incident provided the impetus for the bill's passage. The drug amendments brought about a comprehensive revision in the regulation of new drugs, which at the time included both human and animal drugs. The drug legislation also amended the anticancer clauses to rectify what Congress perceived as the inequity associated with the prior sanctioned use of diethylstilbestrol (DES) in animal feed. Under the Food Additives Amendment of 1958, certain DES uses in animals were prior sanctioned because they were covered by an effective New Drug Application (NDA). Thus, continued use in accordance with the prior sanction was appropriate until that use was cancelled (the NDA revoked), but no new uses in food or food-producing animals were approvable due to the Delaney clause (Refs. 4 and 5).

The act requires that compounds administered to animals as food additives, color additives, or animal drugs be shown to be safe for use. As defined in section 201(u) of the act (21 U.S.C. 321(u)), the term "safe" clearly embraces the health of man, as well as the health of the animals to which the compounds are given. Thus, in evaluating the safety of compounds to be administered to animals raised or maintained for production of food for man, such as cattle, swine, and poultry, Congress has from the beginning recognized that consideration must be given to the safety of possible residues of the compounds in the products of animals that become food for man, i.e., meat, milk, and eggs.

Before 1962, the anticancer clauses in sections 409 and 706 did not distinguish between compounds added directly to human food and compounds that might indirectly enter human food through administration, as feed additives or drugs, to food-producing animals. The act was interpreted as forbidding FDA to approve the use of a carcinogenic animal drug whether or not the compounds might leave any residues in the edible tissues of the animal.

Modification of the effect of the anticancer clause of section 409 had first been suggested during congressional consideration of the Color Addition Amendments of 1960. In May 1960, the then Secretary of Health, Education, and Welfare had urged Congress to modify the act, explaining:

> There is * * * one respect to which the anticancer proviso has proved to be needlessly stringent as applied to the use of additives in animal feed. For example, in the case of various animals raised for food production, certain drugs are used in animal feed which will leave no residue in the animal after slaughter or in any food product (such as milk or eggs) obtained from the living animal, and which are therefore perfectly safe for man. If this is demonstrated with respect to any particular additive intended for animal feed, and the additive will not adversely affect the animal itself during its expected or intended life cycle, we can see no reason for not permitting such a use of an additive which could be highly useful and beneficial in the raising of animals for food. * * *
>
> We therefore have included in the enclosed draft bill an amendment to permit use of an additive in animal feed under the above-mentioned conditions.
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> * * * * *
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> Under the amendment, the assay methods applicable in determining whether there will be a residue shall be those prescribed or approved by us by regulations. This will give reasonable certainty in that regard, although, of course, such regulations may from time to time be changed as new scientific developments demonstrate a need for change. It should be clearly understood that the industry still would have the responsibility of developing adequate analytical methods for detecting residues and furnishing them to the Government with a petition for approval of an additive (Ref. 3).

The amendments proposed by the Department had not been included in the color additive legislation. During the following 2 years, however, concern had been continuing about application of the anticancer clause in section 409. As a result, legislation similar to that earlier recommended by HEW was introduced in 1962. The House Committee on Interstate and Foreign Commerce ultimately included modifications of the anticancer clause in its report on the Drug Amendments of 1962, with the following explanation:

> The committee amended the anticancer clause of the food additives amendment and the color additive amendment of the Federal Food, Drug, and Cosmetic Act by making this clause inapplicable to chemicals such as veterinary drugs when used in feed for food-producing animals if the Secretary finds (1) that under the conditions of use and feeding specified in the proposed labeling and reasonably certain to be followed in practice, such additive will not adversely affect the animals for which such feed is intended, and (2) that no residue of the additive will be found (by methods of examination prescribed or approved by the Secretary by regulations) in any edible portion of the animal after slaughter or in any food such as milk or eggs yielded by or derived from the living animal (Ref. 4).

Representative Leonor K. Sullivan objected to the proviso in the floor debate on the amendments and proposed a separate amendment to delete the proviso from the bill because "they (the provisos to the Delaney clauses) weaken instead of strengthen consumer protection." She reminded the House that DES had been regarded as safe for use in poultry at one

time because no residue was found in the meat; later, that use had to be terminated when DES residues were found as a result of improved testing methods. But her amendment was defeated principally on the argument that, if DES were available for manufacture by those who obtained approvals before 1958, i.e., the prior-sanctioned uses, it should be made available for manufacture by everyone (Ref. 6).

The Senate accepted the modifications of the anticancer clauses in conference while preserving, as Senator Hubert Humphrey noted, the full vigor of consumer protection afforded by Delaney clause (Ref. 7). These modifications have come to be known as "the DES proviso."

4. Animal Drug Amendments of 1968

The animal feed industry experienced an era of unprecedented growth and innovation beginning in the 1950's. That industry and the animal drug industry began an effort in the mid-1960's to consolidate the various provisions of the Federal Food, Drug, and Cosmetic Act governing the premarketing approval of drugs intended for use in animals, i.e., sections 409, 505, 507 (21 U.S.C. 348, 355, and 357) which culminated in the enactment of the Animal Drug Amendments of 1968 (Pub. L. 90-399). Neither the committee reports on the bill nor the floor debates raised the issue of the Delaney clause. Consequently, the Animal Drug Amendments of 1968 passed without controversy and added, under section 512(d)(i)(H) of the act, the following anticancer clause and proviso:

(H) such drug induces cancer when ingested by man or animal or, after tests which are appropriate for the evaluation of the safety of such drug, induces cancer in man or animal, except that the foregoing provisions of this subparagraph shall not apply with respect to such drug if the Secretary finds that, under the conditions of use specified in proposed labeling and reasonably certain to be followed in practice (i) such drug will not adversely affect the animals for which it is intended, and (ii) no residue of such drug will be found (by methods of examination prescribed or approved by the Secretary by regulations, which regulations shall not be subject to subsections (c), (d), and (h)), in any edible portion of such animals after slaughter or in any food yielded by or derived from the living animals, * * *.

Again, the legislative history indicates that the legislation in no way weakens FDA's authority to regulate new animal drugs (Ref. 8).

B. STATUTORY INTERPRETATION

The enactment in 1962 of the so-called DES proviso to the Delaney clause has been a source of continuing controversy. There is no unanimity on the proper interpretation of the proviso; and the legislative history of the proviso, summarized above, does not lay to rest all doubts.

Two interpretations of the proviso are, in theory, possible. The first interpretation, which in the Commissioner's judgment is the less probable, is that Congress intended to allow FDA to approve the use of a carcinogenic compound in food-producing animals only if the agency could be absolutely positive that no traces whatever—no matter how small—would remain in edible tissues.

This interpretation presents several difficulties, all stemming from the fact that any introduction of a compound, whether or not carcinogenic, is likely to leave in edible tissues minute residues, which are below the level of detection of any known or likely to be developed method of analysis, i.e., assay. It is a fundamental fact of analytical science that for every assay developed to measure the concentration of a chemical compound in a medium (in this case, a residue in an edible tissue), there is some lowest concentration or level of the compound below which the assay will not yield an interpretable result (Ref. 9). If, for example, an assay measures a particular compound in muscle tissue, i.e., an edible tissue, and the assay has been shown to have a lowest limit of measurement of 1 part per billion (1 ppb—1 part compound in 1 billion parts tissue on a weight basis, such as 1 nanogram of compound per 1 gram of tissue), examination of muscle tissue using this assay will reveal that the compound is present only if its concentration in muscle tissue is 1 ppb or higher. If the compound is present in the tissue at a level below 1 ppb, use of the assay will yield no interpretable result. Thus, the assay connot distinguish between muscle tissues containing the compound at levels below 1 ppb and muscle tissues from which the compound is absent in the absolute sense of the term.

Although different assays may have different lowest limits of measurement, all assays are subject to the same type of limitation. Thus, when a tissue is examined with an assay having a lowest limit of measurement of 1 ppb and no interpretable response is observed, the analyst can conclude only that the compound under analysis is not present at a level of 1 ppb or above. It can never be concluded that the compound is "not present" in the absolute sense. It is thus impossible to determine the conditions under which edible tissues derived from food-producing animals that have received a carcinogen will contain no residue if the phrase "no residue" is to be interpreted literally. Accordingly, this first possible interpretation of the DES proviso would not permit approving any known carcinogenic animal drug because the Commissioner could never find that no trace whatever would remain in the edible tissues of the animals to which the compound was administered.

This interpretation would thus render the DES proviso a "Catch-22." The proviso would permit the Commissioner to approve carcinogenic drugs for animals only when certain that no residues whatever would remain, but since the Commissioner could conclude only that some trace might well remain, no such drug could ever be approved.

Nevertheless, one comment on the February notice contended that Congress did indeed intend that the no-residue provision be a flat prohibition on any molecules of a carcinogen in food. The comment further argued that Congress did not understand fully the scientific ramifications of its action when it amended the pristine Delaney clause.

As the Commissioner noted in the February notice, the "absolutely no molecules" interpretation seems, at the very least, an improbable interpretation of an amendment enacted by Congress precisely because it wanted to relieve animal drugs from the rigid strictures of the anticancer clauses. Moreover, any interpretation of a statutory provision that would render it totally inoperative should be rejected unless considerations of overwhelming persuasiveness require that interpretation. No such considerations have been advanced in support of the "absolutely no molecules" interpretation of the DES proviso.

Furthermore, this interpretation is difficult to reconcile with the language of the DES proviso itself. It specifies that "no residue" may be "found * * * by methods of examination prescribed or approved by the Secretary * * * in any edible portion of such animals * * *." This language conspicuously avoids such words as "occur" or "remain," and instead, by use of the word "found" emphasizes detectability. Moreover, the same proviso refers to "conditions of use * * * reasonably certain to be followed in practice", suggesting a congressional recognition that some occurrences of these residues (i.e., resulting from unforeseeable misuse) might not require withdrawal of approval of a compound even if they were detected.

A second, and in the Commissioner's view more plausible, interpretation of the DES proviso accepts the words of the amendment and focuses on the previously quoted language, "no residue of such drug will be found * * * by methods of examination prescribed or approved by the Secretary by regulations * * *." Under this interpretation, a sponsored compound that is carcinogenic may be approved for use in ani-

mals if examination of edible tissues by an assay approved by FDA reveals no residues. This interpretation also appears implicit in the limited case law addressing the issue (*Hess & Clark, Division of Rhodia, Inc.* v. *FDA*, 495 F.2d 975 (D.C. Cir. 1974), *Chemetron Corp.* v. *United States DHEW*, 495 F.2d 995 (D.C. Cir. 1974), and *AHI* v. *FDA, supra*).

This second interpretation is in essence the one that FDA has followed since the passage of the DES proviso. The agency has approved carcinogenic compounds for use in animal feed or as animal drugs on the basis of assays capable of measuring prescribed levels of residues.

The court in *AHI* v. *FDA* found lacking the agency's previous attempt to define and explain, as a binding rule, the criteria and procedures for evaluating assays for carcinogenic residues in edible products of animals. The court held that FDA had failed to provide adequate public notice. One purpose of this document is to correct that defect.

The Commissioner believes that the criteria to be applied in evaluating assays for carcinogenic residues in the edible tissue of food-producing animals must further the congressional intent to minimize public exposure to carcinogens, without nullifying the decision reflected in the DES proviso, as the first interpretation of the proviso would do. As explained more fully below, the criteria set forth in these regulations for evaluating assays for carcinogenic residues are minimum requirements. They are designed to identify assays that are (1) reliable and practical for use by a regulatory agency and (2) capable of measuring residues at levels that have been determined, on the basis of animal toxicity tests, to present no significant increase in human risk of cancer. An assay that does not meet both criteria cannot be approved. The Commissioner recognizes that, for some compounds currently in use, no reliable and practical assay capable of sufficiently low limits of measurement now exists and that approval of their continued use must therefore be reexamined.

Arguing that the Commissioner has incorrectly interpreted the Delaney clause, AHI contends that it is a precise statutory provision that must be construed very narrowly. Therefore, AHI charges that the Commissioner's interpretation has unduly, and illegally, broadened the scope of the anticancer clause. AHI contends that FDA must prove that a compound is a carcinogen before the petitioner for the compound's use is required to comply with any provision of the proposed regulations. Ostensibly, AHI argues that FDA must prove that the sponsored compound is a carcinogen before a petitioner is required to submit either comprehensive data from long-term animal studies (the fundamental information for assessing a compound's carcinogenicity), or certain data regarding the residues in food to which man will be exposed if the compound is approved. Also, AHI argues that FDA cannot prevent a sponsor from marketing a compound when any assay for a carcinogen is available, even if the assay fails to exhibit a lowest limit of reliable measurement required by the data and extrapolation procedure proposed in the regulations. Citing *Hess & Clark, Division of Rhodia, Inc.* v. *FDA*, AHI further contends that the Delaney clause imposes upon FDA a standard corresponding to the level of technology at the time the application for the compound (new animal drug application (NADA) or food additive petition) is approved. Moreover, AHI argues that the modified Mantel-Bryan procedure for statistically assessing the risk of chemical carcinogenesis, which was included in the February notice, is a theoretical procedure that would require petitioners to develop assays capable of measuring residues of compounds at levels that are far too conservative and that are technically and economically infeasible. The court in *AHI* v. *FDA* requested FDA to consider AHI's arguments on technical and economic feasibility.

AHI's argument concerning the burden of proof on the issue of carcinogenicity might have merit if the Delaney clauses stood alone and were applied in isolation from the other provisions of the FFDC Act. However, ever since their enactment, the anticancer clauses have been regarded as a particularization of the general safety sections of the act, to which they attach as provisos; and they have been applied in conjunction with the general safety provisions. They do not expand the scope of these sections. Under these general safety provisions, a compound cannot be approved unless it is shown to be safe and in every case the petitioner has the burden of showing safety. Section 409(c)(3)(A) prohibits approval of a food additive if "the data before the Secretary * * * fails to establish that the proposed use of the food additive * * * will be safe * * *." Section 706(b)(4) prohibits the Secretary from approving a color additive "unless the data before him establish that such use * * * will be safe * * *." Section 512(d)(1)B) requires the Secretary to deny approval of a new animal drug if "the results [of tests submitted to the Secretary] show that such drug is unsafe for use under [the conditions prescribed, recommended, or suggested in the proposal labeling thereof] * * * or do not show that such drug is safe * * *." These sections of the act do not impose on FDA any burden to prove that a substance is unsafe. Rather, they impose on the petitioner for approval the burden of showing that, under the proposed conditions of use, the compound is safe.

"Safe" means safe in all respects—including safe from carcinogenicity. Thus, AHI's argument that the burden is on FDA to show carcinogenicity rather than on the sponsor to show noncarcinogenicity is contrary to the clear language of the act. It would impose on FDA two burdens that Congress manifestly intended to impose on petitioners for approval of substances under the act—the burden of testing for safety and the burden of proof on the issue of safety. The Delaney clauses clarify and emphasize the congressional intent to protect the public from carcinogenic risks; AHI would transform them into clauses that reduce the protection from carcinogenic risks already provided by the general safety provisions.

The general safety provisions of the act provide the context for the Delaney clauses. Under them the sponsor of a compound must submit adequate tests by all reasonably applicable methods to show that the sponsored compound will be safe when used. This showing, of course, requires not only toxicity testing but also an assay suitable for measuring the compound and substances formed in or on food as a result of its use. Only after the sponsor of a compound has conducted all the required tests and submitted the resulting data is FDA required to make any showing that the Delaney clause or the DES proviso is applicable or that the compound has not otherwise been shown to be safe.

Adoption of AHI's interpretation that FDA must prove that a compound is a carcinogen before the necessary data are submitted requires an illogical reading of the statute in light of its overall purpose and the legislative mandate surrounding it. Therefore, the Commissioner rejects AHI's scheme of regulating chemical carcinogens and potential carcinogens.

Scrutiny of the *Hess & Clark* decision shows that the court did not even consider the procedure that FDA used to designate requiremens for an assay under the DES proviso to the Delaney clause; rather, the court accepted as valid the agency's designation of an assay. To the extent that the procedures and criteria set forth in this notice for assessing assays differ from those used in evaluating the assay involved in *Hess & Clark*, they are being adopted by rulemaking in an area in which the agency has considerable expertise and discretion because the area involves protecting the public against cancer.

AHI's allegations that the regulations are technically and economically infeasible is an attempt to characterize the agency's actions as arbitrary and capricious. Several environmental statutes (e.g., Clean Air Act, Federal Water Pollution Control Act, Federal

Insecticide, Fungicide, and Rodenticide Act) contain specific provisions requiring the Environmental Protection Agency (EPA) in certain instances to make elaborate cost/benefit calculations in setting safe levels of human exposure to chemicals in the environment. Also, these statutes provide that EPA protect the environment from contaminants by setting standards for the discharges permitted. EPA is authorized to establish two types of standards—health-based standards and technology-based standards. For certain health-based standards the Supreme Court has authorized that agency to require pollution reduction by methods that are neither economically nor technically feasible when the agency is not explicitly required to consider cost (*Union Electric Company* v. *EPA*, 427 U.S. 241 (1976)). The United States Court of Appeals for the District of Columbia Circuit has subsequently reached similar conclusions when interpreting analogous provisions of the Federal Water Pollution Control Act, concerning regulation of the discharge of toxaphene endrin, and polychlorinated biphenyls (PCB's) (see *Hercules, Inc., et al* v. *Environmental Protection Agency, No. 77-1248. (D.C. Cir. Nov. 3, 1978); Environmental Defense Fund, et al.* v. *Environmental Protection Agency*, No. 77-1091 (D.C. Cir. Nov. 3, 1978)).

The two possible exceptions not applicable here (establishment of tolerances for unavoidable contaminants under section 406 and for pesticides under section 408(h)), the Federal Food, Drug, and Cosmetic Act contains no provisions requiring the Commissioner to consider costs or technical feasibility in making any safety decision, including any decision involving cancer-causing chemicals. The distinction between the statutory provisions applicable to food additives, color additives, and animal drugs and those applicable to pesticides and unavoidable contaminant tolerances demonstrates Congress' decision to make costs and technical feasibility relevant to some public health matters but not to others. Nevertheless, in light of the court's remand order, the Commissioner recognized the agency's obligations to review this element of the proposal. Based on the act's legislative history, the case law, and the agency's public protection function, the Commissioner concludes that the procedures used to designate requirements for assays can be technology-forcing if necessary.

The Commissioner's interpret .tion recognizes the tension between the need to provide health protection and the costs of that protection, and it attempts to spur the private sector into technological change only when such change is necessary for protection of the public health. To do otherwise might force the public to accept an increased disease burden that it would unknowingly have to bear. The agency recognizes that the public health is not advanced by imposing requirements for what is neither economically nor technically possible. It also recognizes that public health regulation requires common sense, a sense of proportion, and awareness of economic and technical factors. In particular, the agency should not impose economic costs that are not justified by some reduction of risks to the public health. Nevertheless, the agency can properly require improvements in or developments beyond currently available technology when there is sufficient reason to believe that those improvements or developments are feasible and are needed to protect the public health. In enacting public health legislation, Congress intends that administrative agencies carry out their assigned missions with intelligence, good sense, and an awareness of the context and consequences of their actions; but unless it has expressly said so, there is no reason to think that it intended them to be in thrall to the technological or economic status quo.

In the immediate context, the statutory structure and language provide considerable guidance with respect to the issue of feasibility and costs. The language permitting the use of carcinogenic substances under certain circumstances is a proviso to a clause prohibiting the use of carcinogens, and that clause itself is a particularization of a provision requiring safety generally. It is clear that in enacting the DES proviso Congress intended to create no additional risk of human cancer beyond what would have existed in the absence of the DEX proviso. That is why Congress used the language "no residue * * * will be found." By enacting and twice re-enacting the Delaney clause, Congress made clear its willingness to ban entirely from the human food supply food additives, color additives, and animal drugs that present a carcinogenic risk to man. it enacted the DES proviso with the intent and expectation that the provision that "no residue * * * will be found" would sufficiently protect the human food supply from any significant cancer risk from food additives, color additives, and animal drugs. Thus, in enacting the DES proviso, Congress did not change in any way the policy of the Delaney clause to protect the human food supply from carcinogenic additives and animal drugs; it merely eliminated an application of the clause that it considered unnecessary to the complete achievement of that policy.

From this statutory structure and language, it is evident that any consideration of feasibility and costs is subsidiary to the overriding congressional purpose to permit no additional human cancer risk from food additives, color additives, or animal drugs. The Commissioner's discretion to establish "methods of examination" for detecting residues is to be exercised so as to carry out that congressional purpose. the factor that determines the acceptable level of measurment of an assay method is protection of the human food supply from carcinogenic risks. If, on the basis of toxicological considerations, the Commissioner determines that a certain level of assay measurement is necessary to prevent a significant human cancer risk from use of a carcinogenic substance in food animals, then a method having that level of measurement is necessary to carry out the congressional purpose. If no such method is feasible, or if it is too costly to develop or apply one, then the choice is between refusing to permit the use of the substance altogether and permitting its use despite the fact there is no method of examination that can prevent the use of the substance from presenting a significant human cancer risk. Under the general safety clause and the Delaney clause, that choice can be resolved in only one way: by refusing to permit the use of the substance.

During the last decade, FDA has been monitoring significant trends in the development of chemical, physical, and biochemical methods of analysis of trace toxicants in biological matrices, i.e., tissues, biological fluids, etc. In some cases the agency has examined the available methods, and the trends, of analysis of specific toxicants of public health concern (Ref. 10). In other cases the agency has prepared and submitted to Congress reports on the advancing frontiers of the analytical sciences (Refs. 11 and 12). One of the central findings of this continuing activity is the observation of what can properly be regarded as spectacular scientific progress in achieving ever-decreasing lowest limits of measurement. There is no reason to believe that this progress in analytical chemistry will stop or slacken in the foreseeable future.

Table I shows the trend of the increasing capacity of analytical chemistry to detect the measure the presence of chemicals. Depending on the substance or class of substances, this decrease in the lowest limits of measurement during the last 20 years ranges between two and five orders of magnitude. Table I also suggests that recognition of a public health problem associated with a toxicant accelerates the improvement of analytical methods needed to detect and measure it. In this connection it should be noted that accelerated rates of improvement in analytical methods have generally been the result of public health concerns diffused among the members of the scientific community at large. They have not usually been the result of the concerted effort of a sponsor or industry to gain approval for use of a substance of commercial value.

TABLE I.—*Trends in Analytical Chemistry Detection Techniques*

Compound and date	Detection technique	Limit of measurement	Relative specificity
DDT:			
1940's, 1950's	Colorimetric	10 ppm	Low.
1950's, 1960's	Paper chromatography	1 ppm	Moderate.
1970's	Gas chromatography	Few ppb	Do.
	Gas chromatography/mass spec	Few ppb	High.
Dioxins:			
1940's			
1950's, 1960's	Thin layer chromatography	Non quant	Moderate.
1970's	Gas chromatography/mass spec	Less than .1 ppb	High.
Nitrosamines:			
1940's			
1950's, 1960's	Thin layer chromatography	10-20 ppb	Moderate.
1970's	Gas chromatography/mass spec	2 ppb	High.
Cortisone:			
1940's			
1950's, 1960's	Colorimetric	4 mg/ml	Low.
1970's	High press liquid chromatography	About 5 ng	Moderate.
Chlorpromazine:			
1940's, 1950's	Titrimetric	50-100 mcg	Low.
1960's, 1970's	chromatography	A few mcg	Low.
Hallucinogens (LSD, mescaline):			
1940's			
1950's			
1960's	Colorimetry	mcg/ml	Low.
	Gas chromatography, fluorescence	25 ng	Moderate.
1970's	NMR	Sub ng	High.
Reserpine:			
1940's			
1950's, 1960's	Colorimetry	Greater than 10 ppm	Low.
1970's	Fluorescence	About 5 mcg/ml	High.
Lead:			
1940's	Colorimetry	About 10 ppm	Low.
	Polarography	About 0.1 ppm	High.
1950's	do	do	Do.
1960's	Atomic absorption	About 1 ppm	Do.
1970's	do	do	Do.
Cadmium:			
1940's			
1950's, 1960's	Colorimetry	About 50 ppb	Medium.
1960's, 1970's	Atomic absorption	About 0.3 ppb	High.
Digitalis drug:			
1940's	Bioassay	LD_{50} 80 mg/kg	Low.
1950's	do	do	Do.
1960's	do	do	Do.
1970's	Radioimmunoassay	About 0.5 ppb	High.
Carbamates:			
1940's			
1950's, 1960's	Thin layer chromatography, gas chromatography.	50-100 ng	Moderate.
1970's	Gas chromatography	About 1 ng	High.
Organophosphates:			
1940's			
1950's			
1960's	Gas chromatography	About 40 pg	Moderate.
1970's	do	do	High.

Next, Table II shows the capability of some assays that are currently being used to measure trace contaminants in food. Although the assays have not been evaluated by all the specific criteria proposed by the regulation, they are useful regulatory tools; and the lowest limits of reliable measurement for these assays (which were principally developed by the government for monitoring purposes) illustrate the forefront of current-analytical chemistry.

TABLE II.—*Some Assays for Trace Contaminants in Food That Reflect Current Analytical Capabilities*

Substance under assay	Food	Limit of measurement [1]	Detection and confirmatory techniques	Reference [2]
Cadmium, copper, and lead	Several types	5	Anodic stripping voltammetry [3].	Jones, et al. (1977).
N-Nitrosamines	Several types including meat.	10	Gas-liquid chromatography (GLC); mass spectrometry (MS).	Fazio, et al. (1971); Fine, et al. (1975).
Aflatoxins, B1, B2, G1, G12	Peanut butter	5.0 [4]	High pressure liquid chromatography; fluorescence detector.	Panalaike and Scott (1977).
Benzo(a)pyrene	Smoked foods	2	Thin layer chromatography [5] (TLC) ultraviolet and fluorescence detection.	Howard, et al. (1966).
Aflatoxin M1	Milk	0.1	TLC-fluorescence detection [6]; chemical derivation.	Official Methods of Analysis of the AOAC.
Aflatoxin B1, B2, G1, G2	Peanut butter	5.0	TLC-fluorescence detection [6].	Official Methods of Analysis of the AOAC.
	Corn		TLC chemical derivation	Official Methods of Analysis of the AOAC.
Aflatoxin B1	Eggs	0.1	TLC-flouorescence detection; chemical derivation.	Nesheim, et al. (1978).
Arsenic, selenium, antimony, and tellurium.	Several foods	10 to 20	Atomic absorption; spectrometry; chemical derivation.	Florino, et al. (1976).

TABLE II.—*Some Assays for Trace Contaminants in Food That Reflect Current Analytical Capabilities*—Continued

Substance under assay	Food	Limit of measurement [1]	Detection and confirmatory techniques	Reference [2]
Several chlorinated pesticides	Several foods	30 to 50	GLC-2 different	Official Methods of Analysis of the AOAC.
Tetrachlorodibenzodioxin	Fat, milk, others.	.0001 to .010.	Chromatography high resolution MS (direct probe).	O'Keefe, et al. (1975); Hummell, P. A. (1977).

[1] Parts per billion.
[2] References available from: John Arnold, Industry Information (HFV-226), Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.
[3] Found reliable in interlaboratory validation study.
[4] Sum of all four compounds.

In view of these trends, the Commissioner has examined the general analytical requirements that these regulations will place on animal drug sponsors. Table III below shows the acceptable total level of residues in the diet for representative compounds believed to be carcinogens. These estimated acceptable total dietary levels are derived from bioassay data on the parent compounds alone. The lowest limits of reliable measurement for these compounds that would be required if the compounds were subject to the proposed regulation cannot be calculated in the absence of metabolism data in animals in which a sponsored compound is proposed or intended for use (target aminals). Nevertheless, the values do approximate the limits of measurement that would be required by the regulations and are therefore suitable for comparison with the current analytical capabilities that are shown in Tables I and II. It should be noted that for some compounds the lowest limit of reliable measurement derived from toxicity data may go beyond current analytical capabilities; that it may, however, reflects the technology-forcing aspects of the proposed regulation.

TABLE III.—*Estimated Acceptable Total Dietary Levels of Several Known or Suspected Carcinogens for a Lifetime Risk Level of 1 in 1 Million*

Compound	Reference [1]	Dose [2]
DDT	Tomatis, et al. (1972)	.4
Dimethylnitrosamine	Terracini, et al. (1967)	.95
Ethylene Thiourea	Graham, et al.	2.9
NTA	National Cancer Institute Clearinghouse on Carcinogenesis.	300.0
Vinyl chloride	Maltone (1975)	8.7

[1] Available from John Arnold, Industry Information (HFV-226), Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.
[2] Calculated according to Hoel, et al. (1975) (Ref. 63). (In parts per billion.)

The Commissioner concludes that given the known trends in the development of improved analytical methodology the imposed requirements are attainable at the expense of reasonable effort.

The goal of regulating compounds that are to be used in food-producing animals is to ensure that none is permitted to yield residues in edible tissues at concentrations presenting a risk of carcinogenesis above an acceptable level. This acceptable level of maximum allowable risk (see section V. C. 8 in this preamble) is applied to all carcinogens; thus, equitable treatment of all such substances is afforded by these regulatory requirements. Different carcinogens will require different assay capabilities because of differences in carcinogenic potency. The regulations are designed to require that the lowest limit of measurement of an assay be commensurate with a compound's carcinogenic potency. Because it is not possible to specify the required limits of measurement for carcinogens in the absence of animal bioassay data, it is not possible to ensure in advance that all compounds for which approval is sought in the future will be able to be used in ways that satisfy the requirements of the regulations. It may be that some substances present health risks so great that there is no current technology available that can permit their safe use. In these instances the Delaney clause (including the proviso) requires that the Commissioner not relax health standards in order to approve such substances.

From the information described above, the Commissioner believes that analytical science can meet these regulatory requirements. The Commissioner is not aware of any data to the contrary. Based on this review, the Commissioner has concluded that compliance with the proposed regulations is feasible, although some technological innovation may be necessary.

Questions have arisen about the practicality, efficiency, and overall public protection afforded by automatically adopting new assays that reliably measure lower levels of residues is such assays becomes available after a sponsored compound has been approved for use. In the February notice the Commissioner suggested that this problem is largely theoretical once an assay meeting the minimum criteria is approved. The decision to approve an assay for a sponsored compound under these principles represents the agency's conclusion that the compound has been shown to meet all the statutory requirements of safety. Accordingly, once assay methods have been approved, new methods will not be required without new toxicological data showing that the lowest limit of reliable measurement of residues under these regulations is inappropriate.

It is true that these proposed regulations will permit the approval, for use in animals feed or for use as animal drugs, of carcinogenic compounds that are likely to leave residues below the lowest level of reliable measuremnt of any assay meeting all the criteria of the regulation. Indeed, as a result of Congress' enacting the DES proviso, the agency will not have any certainty that these residues, in amourts below the level of detectability, are not always present. This result makes sense in practical terms, however, for a regulatory agency cannot effectively control residues—of any compound—that are so small that they escape measurement by every available assay. In sum, the interpretation adopted in these proposed regulations is reconcilable with both the purpose and lanaguage of the DES proviso. This interpretation will further the congressional objective of minimizing public exposure to residues of carcinogenic compounds. It does not force technology beyond the point that needs to be reached to carry out the purpose of the Delaney clause and the general safety provisions. It does not impose infeasible requirements or costs except to the extent that they are necessary to carry out that purpose.

C. OVERVIEW OF THE REGULATIONS

The proviso to the anticancer clauses allows the approval of the use of carcinogens in food-producing animals if, under conditions of use "reasonably certain to be followed in practice," no residue is found by an assay prescribed or approved by the Secretary. To ensure public protection consistent with the anticancer and the general safety provisions of the act, the Commissioner must establish criteria for approving assays to include, among other things, an adequate lowest limit of measurement.

Accordingly, these proposed regulations would establish criteria for accepting assays used to measure residues of carcinogens in edible tissues of food-producing animals to which carcinogens have been administered. Such criteria cover assay attributes such as dependability, practicability, specificity, accuracy, and precision. Also, the regulations would establish a specific criterion for the lowest limit or reliable measurement that an assay must meet, as a minimum, before it can be approved by the agency for control of carcinogenic residues. This criterion for the required lowest limit of measurement of an assay derives from toxicological data obtained from carcinogenicity studies and from an operational definition of the no-residue standard of the act. Only if an assay meeting the above criteria is available would the Commissioner have a mechanism to discriminate be-

tween tissue containing a residue and tissue containing no residue. Without such a monitoring mechanism, the Commissioner would have no way to detemine whether a carcinogenic drug or additive administered to a food-producing animal is being or even can be used in compliance with the act.

In these regulations the Commissioner proposes to establish a rigorous premarket testing process for sponsored compounds intended for use in food-producing animals. As proposed, all sponsored compounds must initially undergo a threshold assessment for carcinogenic potential. For those sponsored compounds having a carcinogenic potential, a procedure is prescribed to determine the minimally acceptable lowest limit of reliable measurement for a regulatory assay. Because this limit is determined on the basis of toxicity data, the Commissioner may conclude that an assay satisfying the requirements of the regulations is capable of demonstrating the absence in food of residues that present a risk of cancer to man. By thus particularizing the statutory requirements, the Commissioner proposes to establish the basis for accepting or rejecting compounds which the sponsor claims satisfy the no-residue standards.

1. *Fundamental questions.* For every drug of additive proposed for use in food-producing animals (the sponsored compound), the Commissioner is required by the act to determine whether that sponsored compound can be used in ways that are safe for the animals to which the compound will be administered (target animals) and whether food (meat, milk, and eggs) derived from such animals (edible tissues) will be safe for human consumption. The sponsor of the compound is therefore required to furnish the Commissioner the scientific and technical information necessary for that determination; the Commissioner in turn is required by the act to determine on the basis of all available data whether, in actual practice, the sponsored compound can be used in compliance with the law.

Although a petitioner proposing to use a carcinogenic compound in food-producing animals has a major obligation to develop a practical and reliable assay capable of discriminating tissues that contain residues from tissues free of such residues, as defined operationally, such an assay cannot be developed without certain scientific and technical information.

Specifically, for every sponsored compound, several questions must be answered before an assay can be developed or approval of the compound considered:

a. What is the chemical nature of the sponsored compound and how is it to be used?

b. Based on preliminary toxicological and biochemical information, does the compound have the potential to contaminate human food (edible tissues) with residues of carcinogenic concern?

c. If so, what is the chemical nature of the residues of the compound? in what tissues are they found? at what levels? and for what length of time?

d. Is the sponsored compound or any of the residues it produces in edible tissue carcinogenic in experimental animals or man?

e. If so, what level of residues can be operationally defined as satisfying the no-residue requirement of the act?

f. Can a reliable and practical assay be developed to measure the edible tissue residues at levels equal to or greater than those which operationally satisfy the no-residue requirement of the act?

g. At what time after exposure to the compound ceases do the edible tissues of exposed food-producing animals satisfy the no-residue requirement of the act, i.e., what is the necessary withdrawal time?

2. *Date collection process.* To answer the preceding questions, a petitioner must gather pertinent scientific information, the nature of which is particularized in this document. These proposed regulations would establish the procedure for gathering and evaluating the requisite scientific information. The process is stepwise and evolutionary because the need, as well as ability, to proceed to the next step of data collection depends upon the results obtained at each preceding step. If the evaluation of the data collected at each step indicates that questions on residues of carcinogenic concern remain, data collection must continue. If at some point in the data collection process it can be decided that the sponsored compound presents no human risk of carcinogenesis, the sponsored compound must be evaluated for any other health concerns under the general safety provisions of the act. In this case, the compound may be assigned a safe tolerance level in human food if the petitioner provides the data necessary to establish that the compound can be used safely.

These proposed regulations deal with carcinogenesis, which is a dominant concern in appraising the safety of any sponsored compound intended for use in food-producing animals. Nevertheless, each compound must also be evaluated for other potential adverse effects. Thus, for example, if the available information raises an issue as to the health of progeny, multigeneration studies of the sponsored compound and/or its residues must be codesigned and conducted as part of the process of collection and evaluation of data.

Under this proposal, if the Commissioner makes a threshold determination that a sponsored compound has the potential to contaminate food from food-producing animals with residues whose consumption would pose a human risk of carcinogenesis, the petitioner will be required to undertake the following six-step procedure for data collection and evaluation.

a. A metabolic study in the target animals designed to identify edible tissue residues of carcinogenic concern.

b. Metabolic studies of the sponsored compound in different species/strains of experimental animals designed to aid in selecting the test animal species to be used in chronic toxicity bioassays and in assessing the carcinogenicity of residues that cannot practicably be tested individually ("intractable residues").

c. Chronic toxicity testing to assess the carcinogenic potential of residues of the sponsored compound and to furnish data suitable for statistical treatment so that the no-residue requirement of the act can be applied and implemented.

d. A detailed metabolic study of the sponsored compound in target animals designed to identify both a residue and tissue that can serve as indicators ("marker residue" and "target tissue") to determine whether the no-residue requirement of the act is satisfied.

e. Development of a regulatory assay to measure the marker residue in the target tissue at and above the level established in step d.

f. Establishment of the premarketing withdrawal period required for the safe use of the sponsored compound.

Although the particular provisos to the anticancer clauses of the act, sections 409(c)(3)(A), 512(d)(1)(H), and 706(b)(5)(B), vary slightly in their language, they have a common purpose. Therefore, the Commissioner believes that the criteria for their implementation should be identical. To avoid needless repetition, the Commissioner has used the language of section 512 of the act in discussing specific generic issues because the primary impact of these proposed regulations would be on new animal drugs regulated under that statute. The criteria set forth in this proposal would, however, apply to all chemicals intended for use in food-producing animals, and the appropriate regulations would be amended to adopt these criteria by reference.

II. Threshold Assessment

In the 1973 notice of proposed rulemaking, the Commissioner proposed that carcinogenicity testing not be required for every sponsored compound. Rather, the Commissioner concluded that the necessity for such testing will be dictated by an evaluation of the ex-

isting evidence from metabolic studies, toxicity testing, structural relationships of the sponsored compound and its metabolites to known carcinogens, modes of physiological actions and interactions, and the intended method of use of the sponsored compound.

Comments of two types were received on this feature of the proposal. The first suggested that extensive studies should be conducted from every sponsored compound to determine whether it is a carcinogen. One comment insisted that extensive carcinogenesis testing for every sponsored compound is the only accurate indicator of carcinogenic potential. Several contended that the criteria proposed for use in the threshold assessment were too vague, and objected to the failure to explain how such criteria could be applied in practice. Many other comments agreed with the Commissioner's proposal that extensive carcinogencity testing should not be required for every sponsored compound. These comments recommended that the Commissioner review all available data on a sponsored compound before concluding that the stepwise testing procedure set forth in the proposals should be invoked. Comments of a similar nature were received on the 1977 notice. Furthermore, several comments asserted that the guidelines for the threshold assessment were not specific enough.

The Commissioner agrees that the guidelines for the threshold assessment were insufficiently specific, and the following discussion elaborates the agency's guidelines for conducting threshold assessments.

For every compound intended for use in food-producing animals, the fundamental question to be answered is: "What is the potential that the proposed use of the sponsored compound will contaminate the edible tissue of target animals with residues that engender a risk of cancer to humans?"

When a sponsor starts the process of obtaining approval for use of a compound, it provides to the agency information on matters such as the compound effectiveness and its proposed patterns of use. Often a sponsor will also provide preliminary physiological, metabolic, or toxicological data derived from its own studies or from the scientific literature. At this juncture, the Commissioner believes it necessary that a threshold assessment be made, based on the available data, on the need to proceed to the first of the six steps of data collection required by these proposed regulations. Because entry into the six steps of data collection requires that a petitioner undertake a series of complex and costly experimental studies, the Commissioner concludes that it is not reasonable to demand such studies on a sponsored compound if the preliminary data available justify the determination that public health can be protected without so proceeding.

For the sake of clarity, "the total residue of the sponsored compound" and "residue of toxicological concern" are defined in proposed § 500.83 as follows:

"The total residue of a sponsored compound" means all compounds present in edible tissues of target animals that result from the use of the sponsored compound, including the sponsored compound, its metabolites, conversion products, and any other substances formed in or on food because of the sponsored compound's use. (The term "residue" means any single compound present among the total residue.)

"Residue of toxicological concern" means the total residue minus any constituent residue shown to be safe.

A. GENERAL PRINCIPLES

The threshold assessment is based on the principle that the probability that the use of a sponsored compound will yield edible food animal tissue presenting a risk of human carcinogenesis from residue is the product of the following three factors:

(1) The probability of human exposure to residues that may cause cancer, given the proposed pattern of the sponsored compound's use (Factor 1—Use);

(2) The expected average level or concentration of residues of toxicological concern in the edible tissue of treated target animals under the proposed conditions of use, i.e., when the animals have the potential for marketing as food (Factor 2—Residues of toxicological concern); and

(3) The probable toxicological significance of the residues, based on an assessment of the chemical structure of the sponsored compound, its likely metabolites, and other information suitable for predicting toxicity (Factor 3—Potential toxicological significance).

The threshold assessment functions on the premise that all three of these factors must be considered to answer the fundamental safety question posed above. Under the agency's threshold assessment approach, numerical scores are assigned to the sponsored compound, and each of the three scoring factors contributes to the total score. The following paragraphs describe the scoring system and procedures that can be used to collect data that may lead to information yielding the most reliable scores. By consulting this guideline, sponsors of compounds can assess the status of the sponsored compounds for which they seek approval and may therefore provide relevant and useful preliminary data.

The scoring system uses a value of 1,000 to discriminate between those compounds that will be regulated solely according to the general food safety requirements of the act and those compounds that will, in addition, be subject to this proposed regulation. This system will provide uniformity to the threshold assessment of the risk to the public health from a sponsored compound's residues.

When the only preliminary information available is the proposed pattern of use (factor 1 above), the sponsored compounds will be subject to step 1 of the proposed regulations (§ 500.80(b)(1)(i)). Since without the necessary information FDA must make assumptions that require entry into step 1, petitioners have an incentive to gather pertinent information before approaching FDA.

This decision may be altered or confirmed by subsequent collection of data under these proposed regulations or under the other aspects of the general safety provisions of the act. For example, data collected to satisfy other concerns also covered in the general safety provisions may show that the compound is a potential carcinogen. In that case the compound will be evaluated under these proposed regulations. The obverse is also true.

B. THE SCORING SYSTEM

The total threshold assessment score for a sponsored compound is the product of the values for the three assessment factors.

1. *Factor 1—Use.* The use classification of sponsored compounds is divided into three categories, based on the frequency and extent of the target animal's treatment with the sponsored compound. The use factor is the probability that potentially consumable target animals will be treated with the sponsored compound. (See Table IV.) The values in Table IV represent ratios that approximate the likelihood of human exposure from the proposed use patterns in animals.

TABLE IV—USE FACTOR ASSESSMENT

Frequency and scope of target animal treatment	Score
Administration to individual animals to prevent or treat disease	1
Administration on a herd-wide or flock basis for disease treatment or specific disease prevention (for problem herds or when outbreak of disease has occurred)	10
Administration on a herd-wide or flock basis for production improvement or general disease prevention (e.g., coccidiosis)	100

2. *Factor 2—Residues of toxicological concern.* For this scoring factor, the agency assigns the number equal to the concentration in parts per billion of the total residue of toxicological concern occurring in the edible tissue that is the most efficient accumulator of residues in the target animals at the earliest time the animals

are expected to be marketable as food. Without total residue data, the sponsored compound will automatically be required to proceed to step 1 in proposed § 500.80(b)(1)(i).

Lacking information on the composition of the total residue in the edible tissues, the agency must assume that the total residue is of toxicological concern. The score value may be lowered if the sponsor gathers information on the composition of the total residue. For example, a sponsor may demonstrate that a portion of the total residue is a compound for which adequate studies have already been conducted to show that its presence as a residue is not of human health concern.

3. *Factor 3—Potential toxicological significance.* The values for scoring factor 3 reflect the agency's concern that the residues resulting from use of the sponsored compound are likely to cause cancer. The value will be obtained by taking into account available information concerning the potential toxicological activity of the residues themselves or of structurally related compounds, and compounds related by common physiological activity. The Commissioner recognizes that structure/activity relationships and the short-term biological tests discussed later have not been sufficiently developed to permit definitive predictions of carcinogenic activity (Refs. 13, 14, and 15). Nevertheless, the Commissioner believes that they can make a contribution to the threshold assessment.

In the following paragraphs, three sources of information on the basis of which the third factor is scored are discussed: (a) Structure/activity relations; (b) short-term screening tests for carcinogenic potential; and (c) other biological, physiological, and pharmacological data.

The possible values for scoring factor 3 are 1, 10, and 100. A score of 100 is assigned if there is evidence from any of the three sources of information that raises a suspicion that the residue is carcinogenic. A score of 10 is assigned if short-term screening tests for carcinogenic potential have not been conducted and there is no basis for suspecting carcinogenic activity based on the other sources of data.

A score of 1 is assigned when a battery of short-term screening tests for carcinogenic potential has been conducted, when the results show no reason to suspect carcinogenesis, and when there is no suspicion of a carcinogenic potential raised by the other information sources.

(a) Structure/activity assessment: FDA maintains a list of structural characteristics that can be used as a guide in initially determining when, based on structure alone, there may be concern about carcinogenic potential. The list includes all structural types for which one or more compounds have been shown to produce cancer in animals or man. Specific functional groups, e.g., aromatic nuclei, are included where there is evidence that these groups are the dominant influence in carcinogenic potential (Ref. 16).

Because new information is rapidly gathering in this area, the Commissioner expects the FDA list to be updated frequently and recognizes that this list is not exhaustive. An FDA committee on structure/activity relationships will provide an in-depth evaluation of substances with structural features found on the list before a final score is assigned.

(b) Screening tests for carcinogens: Evidence about the validity and utility of short-term in vitro tests as tools for regulating chemicals is growing rapidly. The Commissioner has concluded, however, that they cannot be used as the principal tool in assessing the safety of a compound. An appropriate battery of such tests can provide useful but not conclusive information about the safety of chemicals quickly, and at a reasonable cost. For these reasons, the Commissioner has included this section in the preamble as a guide to using these tests.

Currently, an appropriate battery of short-term tests includes both mammalian and nonmammalian test systems. The battery should test the ability of a sponsored compound to induce point mutations in two test systems that have been demonstrated to have a high correlation between detected mutagens and positive results in in vivo carcinogenesis bioassays. Systems that have shown this correlation include (1) point mutations in bacteria, (2) point mutations in the X-linked recessive lethal test in Drosophila, and (3) point mutations in mammalian cells in culture. Unscheduled DNA repair synthesis in mammalian cells in culture should also be included in the battery.

There is extensive literature correlating results in bacterial mutagenicity tests and carcinogenicity as determined by chronic toxicity studies (Refs. 17 through 20). This correlation is not perfect, and certain classes of carcinogens cannot be detected in mutagenicity assays.

The published data on mutations and DNA repair in eukaryotic cells are not as extensive as data concerning the Ames bacterial mutagenesis tests. The tests in mammalian cells appear to complement those in bacterial cells for the correlation of mutagenicity and carcinogenicity (Ref. 21). Testing in other systems is particularly important when the chemical is toxic to bacteria, as are many animal drugs, especially antibiotics. This toxicity will often make it impossible to test the chemical at a sufficiently high dose for negative results in bacterial tests to be meaningful.

All short-term tests for carcinogenicity should be performed separately in the presence, and in the absence, of a metabolic activation system, generally derived from rodent liver or the liver, or other relevant tissue, of the target animal. When appropriate, metabolites should be treated with glucuronidase and aryl sulfatase before testing.

Due to the rapid advances being made in the field (Refs. 22 through 34), it would be inappropriate for this proposal to prescribe or recommend detailed protocols for each general type of test. At the present time the most reliable, perhaps the best, results are obtained with the plate incorporation assay described by Ames (Ref. 22).

Application of the screening tests for scoring factor 3 requires some knowledge about the composition of the total residue to determine which residues should be subjected to the complete battery of tests. Although the sponsored compound should always be subjected to the complete battery of tests, for some or all metabolites it may sometimes suffice to perform less extensive testing, e.g., bacterial testing only. The sponsor should explain the reasons for selecting certain metabolites for testing and the reasons for not testing others. Similarly, use of an incomplete battery of tests should be explained. Factors such as structure and residue concentration in tissue should be addressed. In addition, a reduction in testing for any major metabolites should be justified based on factors such as the structural relationship to more exensively tested compounds.

Because of evidence that some structural classes of carcinogens may not yield a positive response in the short-term tests, there will be cases when results from such tests cannot be accepted.

(c) Other biological and pharmacological data: The sponsor should provide the results of a literature search on the sponsored compound and postulated metabolites. This search should also include relevant information on biological activity of structurally related compounds, particularly when very little information is available on the sponsored compound. The sponsor should also include and discuss any relevant information on the pharmacologic and physiologic activity, such as studies that may provide clues regarding the mode of action and expected toxicity. Frequently, in support of the investigational use for the chemical, the sponsor will have gathered some information on pharmacolo-

gic and physiologic activity and will also have developed subchronic test data in experimental animals, e.g., 90-day rodent and nonrodent studies. The data must be submitted for incorporation in the threshold assessment.

The foregoing types of information will be analyzed in the threshold assessment to identify any evidence suggesting that the sponsored compound or its expected metabolites is carcinogenic. This evidence will include findings of hyperplasia or of an abnormal proliferation of any type of cells. These findings lead to a suspicion of carcinogenic potential because such changes have frequently been shown to progress to cancer in studies of longer duration. Also, suspicion is raised by evidence of liver or kidney necrosis and evidence of the formation of regenerative nodules. Certain endometrial changes may also be indicative of possible preneoplastic effects (Ref. 35).

Other examples of biological information raising a suspicion of carcinogenic potential of a compound or its metabolites are binding to cellular nucleophiles, or an indication of the alteration of nucleic acid. Estrogenic compounds will be considered to be suspect carcinogens. Any compound that has the ability to disturb normal hormonal balance, a fact that may be known from pharmacologic studies, or that may be suspected from the organ effects observed in short-term toxicity studies, will be of carcinogenic concern.

4. *Scoring system and the threshold decision.* After the threshold assessment has been completed, each compound is assigned a scoring number that is determined by multiplying score factor 1 (use) times score factor 2 (amount of the residue) times score factor 3 (structure/biological activity). A compound with a score number above 1,000 raises enough concern about the potential contamination of food with carcinogenic residues that is must at least enter the first step of data collection specified by the regulations. The data collection process for a sponsored compound receiving a score equal to or less than 1,000 begins in accordance with the requirements (for risks other than cancer of the general safety provisions of the act. If, at any time after this data collection process begins, the data show that the risk of cancer is greater than that indicated by the threshold assessment score, the sponsored compound will become subject to these regulations.

Table V below shows the maximum concentrations of total residues of toxicological concern that could be found in the most efficient accumulator among the edible tissues and the corresponding scores of factors 1 and 3 that together would permit a sponsored compound to be exempt from the requirements of the regulation.

TABLE V—THRESHOLD ASSESSMENT*

Use (factor 1)	Residue maximum (factor 2) parts per billion	Structure/ biological activity (factor 3)
1	1,000	1
1	100	10
1	10	100
10	100	1
10	10	10
10	1	100
100	10	1
100	1	10
100	0.1	100

*Maximum concentration of total residue of toxicological concern that could be found in the most efficient accumulator among the edible tissues and the corresponding score of factors 1 and 3 that would permit sponsored compounds to be exempted from the regulations.

III. METABOLIC STUDY IN TARGET ANIMALS TO IDENTIFY RESIDUES OF CONCERN

A. NEED TO IDENTIFY RESIDUES IN EDIBLE TISSUES

Before any decision can be made concerning conditions of safe use of a sponsored compound, it is necessary to obtain information on the residues that occur in edible tissues when the compound is administered to the animals for which it is intended (target animals). Without such information, informed decisions about human safety regarding edible tissues derived form treated animals are not possible.

A substance administered to target animals is not necessarily the substance consumed by persons who eat the edible products of target animals. The enzymatic system or physiological fluids of an animal can act upon a compound administered to the animal and produce new compounds in the process (metabolites and degradation products of the sponsored compound). Therefore, the sponsored compound is not the only tissue residue of concern. Sections 512(b)(7) and 512(d)(2) of the act explicitly provide that, before approving its use, the Commissioner must consider the safety of any substance formed in or on food by a sponsored compound. The toxicity of substances derived from a sponsored compound (metabolites and degradation products) is not necessarily of the same magnitude and type as the toxicity of the parent compound, i.e., some metabolites may be considerable more toxic and some considerably less toxic (Refs. 36, 37, 38). Moreover, metabolites of the sponsored compound that were at one time considered "detoxification" products of the target animals (e.g., glutathione conjugates, mercapturic acid conjugates, and sulfates) actually may represent a hazard when consumed by humans (Ref. 38).

Numerous comments were received on the requirements of the 1973 and 1977 notices for metabolic studies. Several comments stated that no attention should be paid to metabolites. Other contended that metabolism studies should not be routinely required, on the ground that the pathway of excretion is of no toxicological importance if all the administered compound has been eliminated from the tissues of the target animal. Most comments recommended that a metabolism study be required only to determine the major metabolites in the edible tissue of target animals; they suggested that the public health would not be served if sponsors were required to pursue endless structural elucidations and quantitations of all metabolites even though some of them might constitute minor fractions of the total residue of the sponsored compound. Comments also contended that it may not be experimentally possible to administer to animals sufficient quantities of a compound to obtain adequate amounts of residues for structural identification. Several comments asserted that studies should be limited to identification of residues in the edible tissues of target animals and that generally it would be unnecessary to have this information on metabolites in inedible tissues. Further, some comments stated that radiotracer studies can be employed to determine the time by which the sponsored compound and its metabolic products are eliminated ("out time"). However, many other comments suggested that all metabolites be identified and tested for toxicity.

The Commission reiterates that metabolic studies are necessary to assure that sufficient information on residues is collected to permit a food safety evaluation, which in turn can be used to establish criteria for regulatory assays. Therefore, the Commissioner has concluded that the metabolic studies discussed below in this preamble are necessary to determine whether the proposed use of a sponsored compound is safe. Also rejected are the arguments that the agency can consider, under the Delaney clause, only the carcinogenic potential of the sponsored (parent) compound. The Commissioner concludes that industry argument that metabolites of the sponsored compound are excluded from regulation under the Delaney clause and covered only by the general safety provisions of the act rests on a strained reading of the act, which ignores the language and purpose of the Delaney clause. A substance may properly be said to induce cancer when it or any substance which it may become through metabolism induces cancer. Consequently, in determining whether a substance induces cancer, it is appro-

priate—and in accordance with the congressional purpose of protecting the human food supply from added carcinogens—to examine metabolites as well as parent compounds.

Further, even if the Delaney clause were inapplicable to metabolites, the general safety standard would still apply, i.e., it imposes the same requirements that the Delaney clause imposes. So even if the industry argument were correct, it would not change the regulatory outcome. Nevertheless, the industry argument also illustrates that the general safety provisions encompass the anticancer clauses of the act. Assessment of a compound's safety requires a comprehensive examination of the sponsored compound and all of its metabolites and breakdown products. To the extent that the language in § 514.1 (21 CFR 514.1) implies a different view, the Commissioner is proposing to reword that regulation to correct any possible misunderstanding.

B. CONDUCT OF METABOLIC STUDY

1. *Test animals.* The metabolic fate of an administered compound in an animal may be unique for each livestock production class. Therefore, the Commissioner concludes that a metabolic study in the animals for which a sponsored compound is intended (target animals) is necessary. If the petitioner can demonstrate that the data from the metabolic study obtained for one production class are applicable to a second, the Commissioner may modify the extent of the investigation required for the latter.

2. *Required technology.* The metabolic fate of a compound administered to food-producing animals is pivotal in determining the need for and extent of carcinogenesis testing. It is mandatory that the metabolic fate be adequately determined. It is necessary that residues of potential carcinogenic significance have been detected at levels obtainable by the best analytical technology available. Therefore, the Commissioner concludes that the required metabolic studies must be conducted with the best analytical methods that technology provides.

As set forth in part VI of this preamble, one residue must be selected to serve as a practical indicator to assure that the "no-residue" standard of the act is met. This residue can be selected only by reference to a metabolic study in which residues are detected and measured at levels dictated by the outcome of actual carcinogenicity testing. Because these levels cannot be known at the outset of this phase of the metabolic study in target animals and because the "best available technology" may not be adequate to measure the levels dictated by the outcome of carcinogenicity testing, it may be necessary to develop improved technology and to repeat the metabolic study in target animals after carcinogenicity testing has been completed. Another requirement of the second metabolic study will be the collection of enough data to construct tissue concentration-time profiles for some residues.

3. *Analytical techniques.* For the foreseeable future, the general technique of choice for metabolic studies will be the use of radiotracers. The proposed regulations, therefore, consistent with principles that assure scientific quality, recommend that the required metabolic studies be conducted with radiolabeled compounds of the highest specific activity available. These principles concern the types, the chemical nature, the chemical and metabolic stability, and the suitability of radiolabels for metabolic studies having specific objectives. The principles have been developed from past metabolic studies with radiotracers, and adherence to them ensures the scientific quality of the required metabolic studies (Refs. 39 and 40).

The task of residue detection can often be made easier by available information on the metabolism of related compounds. It is recommended that proposed metabolic pathways which appear applicable to the sponsored compound be based on relevant literature references about compounds of similar structure. This information can usually simplify the choice of radiolabel positions, which will ensure that all residues containing structural moieties of potential toxicological concern can be detected. However, these projections of likely metabolism can never be a substitute for experimental observation of the metabolic fate of the sponsored compound.

Although use of radiotracers is the preferred experimental procedure, some compounds possess inherent physicochemical characteristics (e.g., strong fluorescence associated with the structural moiety of potential toxicological significance) that will allow the necessary detection of residues. In such cases, the use of radiolabels may not be required.

4. *Dose regimen.* The dosing regimen for the metabolic study in the target animals must be consistent with the maximum proposed use level and duration of exposure to the sponsored compound. For compounds administered continuously over long periods of time, administration for the metabolic study need continue only until equilibration or saturation of edible tissues has been demonstrated. If tissue equilibration cannot be shown, the sponsor must show that the pattern of residues has stabilized.

The metabolic fate of a compound administered to target animals is likely to depend on the conditions (level, method, and duration) of use (Refs. 41 and 42). Because the purpose of the required metabolic studies is to characterize and quantitate residues under conditions of proposed use, these conditions must be followed in the metabolic studies. However, it is possible that under these conditions certain residues are produced in amounts that do not allow extensive chemical characterization. If the structure of any such residues must be determined, and if sufficient amounts of residues can be produced by administering larger doses of the sponsored compound to target animals, the petitioner would be allowed to follow this procedure. In some instances, chemical synthesis of residues may be easier.

5. *Required date.* Because the relative persistence of residues in edible tissues (i.e., the likelihood that residues will be found in edible tissue) is one consideration in selecting specific residues for toxicity testing, the proposed regulations require that the total number and the relative quantities of residues be determined immediately following cessation of treatment, as well as at a sufficient number of intervals after the initial measurement to determine the depletion trend of individual residues. The number of these measurements needed to identify depletion trends depends upon the kinetics of depletion of the sponsored compound, and for this reason the complete extent of data collection cannot be specified in advance.

The need for, and extent of, chemical characterization of residues depends on a number of factors. Ordinarily, compounds constituting a significant fraction of the total residue require sufficient physical and chemical characterization to permit a determination of whether or not a structural change has taken place that could increase the carcinogenic potency of the residue over that expected of the sponsored compound, e.g., formation of epoxides from olefins, N-hydroxylation of aromatic amines, cyclization of hydroxyacids to suspect lactones (Refs. 14 and 15). In some instances, it may be impossible to judge whether the residue has carcinogenic potential, but sufficient structural alteration alone may be enough to establish the need for further characterization. Because these structural changes are common during metabolism and because it is the tissue residues to which human beings potentially will be exposed, this characterization will normally be required. When the agency determines that a component of the residue requires chronic toxicity testing (because of tissue concentration and persistence and/or expectation of increased carcinogenic potential), chemical characterization and an effort to obtain sufficient quantities of

the residue(s) for toxicity testing will be necessary. (See, however, section III.C., below.)

Residues that appear to become "bound" to tissue components (i.e., those whose rate of depletion appears to be no greater than the turnover rates of tissue components) cannot be automatically exempted from the requirements of the regulation. These residues may be hazardous to humans ingesting edible tissues. The residues can be identified by a variety of standard techniques (Refs. 44, 45, and 46). Of course, any such residue will be exempt from the regulation's requirements if it can be shown that it is a normal tissue constituent deriving from a metabolite of the sponsored compound that has entered normal pathways of intermediary metabolism of target animals (Ref. 43).

In some instances, a sponsor may be required to pursue the complete characterization of certain relatively minor metabolites if partial physiochemical characterization indicates that a structural change during metabolism in the target animal has introduced molecular moieties of carcinogenic potential greater than that expected of the sponsored compound, e.g., nitrosation of an amine of unknown carcinogenic potential to produce nitrosamines of known carcingenic potential (Refs. 14 and 47).

Because uncharacterized tissue residues may pose a risk to public health, the proposed regulation would require that the procedures for separation, purification, characterization, and identification be consistent with the best available scientific and technological capabilities. Ordinarily, the agency will require attempts at characterization to include use of a variety of procedures based on the various forms of chromatography, spectroscopy, and spectrometry.

Allegations have been made that the regulations impose unreasonable requirements (i.e., that the regulations require inordinately complex, and therefore costly, experimental procedures) and that the information to be gained from these tests is not worth the costs of gathering it. Both allegations either ignore the current state of these sciences or misunderstand the requirements of the proposed regulations. All the procedures described in the proposal are standard techniques that are widely used in basic biochemistry and pharmacology investigations. A few comments showed confusion about the requirements associated with the metabolite identification study. To correct any potential misunderstanding, the Commissioner has eliminated the earlier requirement that all residues of the sponsored compound be identified until the sponsored compound has been depleted for three half-lives in the target animals. A safety assessment requires information on the trends of residue depletion in the target animal's tissues. Therefore, the Commissioner proposes to substitute the requirement that residues be identified at sufficient intervals to permit determination of the trends of depletion of individual tissue residues.

6. *Format for data submission.* The Commissioner has concluded that the format for presenting results of metabolic studies should be standardized to minimize the possibility of misinterpreting the data. Because these studies will be the basis for major public health decisions, the Commissioner considers it essential that they be carried out and reported in keeping with the best available criteria. The two professional societies listed in the proposed regulations (American Chemical Society and American Society of Biological Chemists) follow policies for accepting manuscripts that embody the best available criteria for collecting, interpreting, and reporting scientific data of the type required by this regulation.

C. COMPARATIVE METABOLISM STUDY TO AID IN ASSESSING CARCINOGENICITY OF INTRACTABLE RESIDUES

1. *Sponsored compound always tested: rationale and procedure.* The sponsored compound itself must always be tested for carcinogenesis when it is determined on the basis of the threshold assessment and the initial metabolism study required by the regulation that a sponsored compound has the potential to contaminate edible tissues with residues whose consumption may pose a human risk of carcinogenesis. Even if the sponsored compound is not detected among the residues, there are compelling reasons for testing the sponsored compound in addition to testing any residues identified according to the criteria already discussed in section III.B above. Metabolic transformation or nonenzymatic degradation of a sponsored compound can lead to a number of tissue residues that cannot be obtained (either by isolation or synthesis) in sufficient amounts for carcinogenicity testing. (These residues are referred to in this document as "intractable residues".) Testing the sponsored compound itself, therefore, provides an experimental means for acquiring data bearing on the carcinogenic potential of such residues.

Although the dominant criterion for selecting test animal species or strains for chronic toxicity testing will be the degree to which a species or strain models man, applying a secondary criterion for selection can help to address the problem of intractable residues. Specifically selection of test animals can also be based on comparative metabolism data (target animal versus test animal). These data can be used to determine the extent to which particular species or strains, due to the way they metabolically convert the sponsored compound, will be exposed during testing to the same complement of residues to which man may be exposed in tissues derived from target animals.

For example, if a metabolite detected as a residue in edible tissues of the target animal is determined to be toxicologically important, the sponsor will be asked to isolate or synthesize the compound for purposes of toxicity testing. If all such attempts fail, then the comparative metabolism approach is available if a potential test animal species, when adminstered the sponsored compound, is shown to produce the same metabolite. There is thus some assurance that the toxicity test of the sponsored compound also provides an estimate of the toxicity of the intractable metabolite. Because human food could be contaminated with the intractable metabolite, this test is a practical approach to a complex and important issue.

This construct was included in the February 1977 notice in response to comments that either suggested that all metabolites ought to be ignored (which the Commissioner concludes is neither legally nor scientifically acceptable) or that all metabolites must be isolated and independently tested (which is not always possible, for technical reasons). Further, the Commissioner invited additional comment on this construct.

Comments on the use of comparative metabolism to deal with intractable residues addressed several points: the definition of "intractable residues," the criteria for determining whether a test species will produce the same complement of intractable residues as the target animals, the basis for treating tractable and intractable residues differently for chronic testing, and the potential use of "relay" toxicity testing.

One comment misinterpreted the definition of "intractable residues." It suggested that they are substances about which nothing is known. The regulation, however, proposes to define the term "intractable residues" as those that either cannot be isolated from biological material or cannot be synthesized for purposes of further testing. The experiments that will already have been conducted for determining the presence of intractable residues (e.g., chromatographic and spectroscopic experiments) will furnish considerable information about the physical and chemical characteristics of the residues. Accordingly, basic techniques of biochemistry and phar-

macology can determine whether the test animal species will be exposed to the same complement of residues that appear in the target animals' tissues. These techniques will ordinarily supply enough information to make such an evaluation. Therefore, the Commissioner concludes that the comparative metabolism studies have merit for the purpose of dealing with intractable residues.

The Commissioner established a series of requirements that can be satisfied by different experimental techniques having varying degrees of rigor. To avoid multiple interpretations of the same set of experimental observations, the Commissioner concluded that there must be established an additional general requirement that the experimental technique with the greatest degree of rigor be the one used for metabolic studies, and the agency adopted the term "best available technology" to describe this requirement. Thus, if the nature of residues can be determined by ultraviolet spectroscopy (a method of very low specificity) or by mass spectrometry (a method of high specificity) the Commissioner will require the use of the mass spectrometric method.

The Commissioner rejects the suggestion that all compounds be treated as the intractable residues are. Animal bioassay of specific metabolites is the best method of determining potential for chronic toxicity, and the Commissioner would prefer to have all metabolites chronically tested. However, recognizing the limitations of organic synthesis, separation sciences, and facilities available to conduct long-term bioassays in animals, the Commissioner has settled for using comparative metabolism for safety assessment of those residues requiring the application of techniques beyond the bounds of the best available technology. Nevertheless, sponsors will be held to the task of conducting the best type of toxicity study for selected residues that are susceptible to identification and isolation, or synthesis, by the best available technology. Although deeming it essential that sponsors pursue those goals with the best science and technology available, the Commissioner recognizes that the somewhat less than ideal toxicity assessment rendered by the comparative metabolism approach is useful for intractable residues. This position is a reasonable balance between completely ignoring all intractable residues and requiring their pursuit in the absence of the necessary technology.

One comment suggested feeding to test animals the contaminated tissues from treated target animals to assess the safety of residues to which humans will be exposed ("relay" toxicity testing). The Commissioner rejects using relay testing because it has two important limitations. Practical animal testing is limited to a relatively small number of animals as surrogates for the entire human population, and the only way to overcome the known limitations of such bioassays is to feed the small number of animals levels of the test compounds that are far in excess of the levels of animal drug residues to which humans are expected to be exposed. Because tissues of animals do not contain residue levels sufficiently high to compensate for the known limitations of standard bioassays and because they therefore are not a suitable basis for evaluating the residue's carcinogenic potency, as that term is used in this notice, the Commissioner must reject the use of relay toxicity testing. Further, the direct use of tissues from treated animals as test material does not permit determining which, if any, specific residues are responsible for the observed effects and the contribution of the residues to the effects.

Data collected according to the procedures and criteria above will: (i) Establish the number of metabolities in target animals and in a number of species/strains of test animals; (ii) provide information about the chemical structure of these metabolities (the structure of some metabolities will be known completely although for others only partial information will be available); (iii) provide information about the persistence of these metabolities in tissues; and (iv) provide information about their mutagenic, cell transformation, or their DNA damage potentialities. This information will permit FDA to classify the residues into the tractable and intractable categories, to select from the category of tractable residues those that must be subjected to chronic toxicity testing, and to document this selction. Criteria for classifying residues into the tractable and intractable categories were discussed earlier. Criteria for selcecting tractable residues for chronic toxicity testing will be discussed in turn below.

First, it is unnecessary to require that all tractable residues be subjected to chonic toxicity testing. Most often, judicious use of well-established biochemical knowledge will eliminate the need for such extensive testing. A good estimate of the carcinogenic potential of the sponsored compound and its metabolities can be obtained without testing each of the tractable metabolities.

Ordinarily, xenobiotics are metabolicly transformed by target animals, test animals, and man in sequences of enzyme-catalyzed reactions, with considerable interspecies similarities (Ref. 48). The described metabolic studies, especially the studies in comparative metabolism, will provide significant information about these reaction sequences and there interspecies similarities.

It is obviously unnecessary to subject to independent chronic toxicity testing intermediates in sequences that are reasonable expected to be similar in man and the selected species of test animals, and which also are residues in target animal tissues. Testing the leading substrate of each sequence will be sufficient. Tractable residues in target animals that are not produced by the selected test animal species must be tested independently in the absence of information that they are not carcinogenic.

Finally, to estimate reasonably the carcinogenic poetential of the sponsored compound and its metabolites in target animal tissues, one must eliminate the confounding effects of metabolites or sequences of biotransformation reactions unique to the chosen test animal species. These metabolities, if present, could be subjected to short-term tests (mutagenicity, cell transformation, or DNA repair) to assess their inherent potential to produce irreversible effects when in intimate contact with tissues and tissue components. Negative findings would eliminate these residues from further consideration as factors likely to confound the results of bioassays. Further, if these residues are known or expected to be common to the chosen test animals and man, negative findings would eliminate them from the residues of toxicological (in this instance carcinogenic) concern. On the other hand, a positive finding would be a clear indication that they are prime candidates as the causative agents of adverse findings in test animals.

2. *Selection of residues for chronic toxicity testing.* Based on all the studies described above, the Commissioner will select those residues, in addition to the sponsored compound, that require chronic toxicity testing.

IV. Chronic Toxicity Testing

The sponsored compound and any residues selected for testing must be subjected to oral, lifetime, dose-response studies in two of the test animal species strains selected under the criteria described in the foregoing paragraphs. The purpose of these studies is to determine whether the compounds under test are carcinogenic and, if so, to establish the lowest limit of reliable measurement that must be achieved by any regulatory assay for monitoring residues resulting from use of the sponsored compound.

Several comments on this feature dealt with testing chemical compounds for carcinogenic potential, and addressed two major issues: (i) The design of chronic studies, and (ii) the

relevance of animal testing in evaluating human safety.

A. DESIGN OF CARCINOGENICITY STUDIES

Comments on the proposal and the notice expressed contrasting opinions on the design features of carcinogenicity studies with experimental animals. The comments specifically addressed: (i) Selection of appropriate test animals; (ii) conditions, levels, and duration of exposure; (iii) statistical design as it relates to number of animals assigned to the various levels of exposure; and (iv) the adequacy of controls.

The impact of these design features on interpreting animal carcinogenesis data is an important and controversial matter currently under intense scientific investigation. The major effort at FDA's National Center for Toxicological Research (NCTR) is specifically aimed at developing relevant protocols and experimental designs for carcinogenicity testing. The agency has also begun to work on supplementing the NCTR effort within the Interagency Regulatory Liaison Group (IRLG). Until these efforts are concluded and the results incorporated into regulations or into official publications, the Commissioner recommends as guidance the report of the Food and Drug Advisory Committee on Protocols for Safety Evaluation: Panel on Carcinogenesis, Report on Cancer Testing in the Safety Evaluation of Food Additives and Pesticides ("Toxicology and Applied Pharmacology," 20:419–438, 1971). This report reviews and analyzes all facets of experimental design that have been developed and scrutinized by competent scientists before 1971. To facilitate incorporating later developments in testing standards as they evolve, the proposed regulations suggest that petitioners submit developed protocols to the Commissioner for review and updating before initiating studies.

B. RELEVANCE OF ANIMAL TESTING IN EVALUATING POTENTIAL FOR HUMAN CARCINOGENESIS

Several comments on this aspect of the regulation dealt with the merits and shortcomings of animal testing as an experimental tool. Some comments pointed out that even animal testing using the best experimental protocols can never prove conclusively that a compound is not carcinogenic, and that under these circumstances some weak carcinogens may escape identification. Other comments expressed the contrasting view that adequate protocols can be devised. Still others questioned the propriety of drawing conclusions about human carcinogenesis from data collected with experimental animals. Additional comments of the same type were received on these issues after the February 1977 notice. None of these comments provided any evidence or argument that persuades the Commissioner to revise any provision of this part of the regulations. Several comments sugested using short-term in vitro tests, singly or as part of a tiered testing system, as a substitute for long-term toxicity testing. One comment stated that the regulation should apply only to directly acting carcinogens and that indirectly acting carcinogens should be treated differently.

The act requires that in assessing the safety of animal drugs the carcinogenic potential of residues be evaluated. Ordinarily, the evaluation must be based on appropriate testing. Given the gravity of the decisions that depend on the results of these evaluations, the most relevant scientific information must be collected. As a source of information, direct carcinogenesis testing of chemical compounds in man is and must remain beyond the ethical bounds placed by society on human experimentation. Without this information source, which would be the most relevant, alternative sources are human epidemiology studies and animal experimentation. Human epidemiology may provide post facto information about the carcinogenic effects of chemical compounds on man. However, this experience cannot be the central basis for food safety valuations for several reasons, including the inherent imprecision of human epidemiology and the same ethical objections that make direct experimentation in man unacceptable.

There may be a high degree of confidence that a compound found to be a carcinogen in an epidemiology study is a human carcinogen because no interspecies extrapolation is required. However, so-called "negative" epidemiology data (data not showing carcinogenesis associated with a substance) are generally inadequate to overcome positive evidence of carcinogenesis from an animal study. Sources of data are often inadequate for identifying a specific exposed human population. Human beings are exposed to multiple potential carcinogens, and it is difficult or impossible to distinguish their several effects. Moreover, the precise amount of human exposure to particular substances is rarely known. Thus, limitations on the use of epidemiology data include (1) the degree to which the study population can be defined in terms of potential exposure, number exposed to the suspected risk, and the length of the observation intervals, (2) the degree to which the "standard" population used as the control is comparable to the study population, and (3) the role of other factors that might be related to different carcinogenic responses. Further, seldom are there sufficient numbers of subjects available to permit broad-scale conclusions.

The degree to which study populations can be characterized by the level of exposure to specific carcinogens will ordinarily vary considerably because of the lack of measurement in the early years of exposure. Comparison of exposed populations requires contrasting morbidity and mortality statistics of a target population with those of a "standard" population. However, the validity of any conclusion reached from these comparisons depends upon the extent to which other variables related to cancer incidence can be matched, adjusted, or accounted for in the analysis. These controls on data are costly, time consuming, and fraught with imprecision. Finally, detailed human pathology, which is important in demonstrating the role of specific carcinogens in the induction of rare tumors, is seldom available.

The Commissioner therefore concludes that the agency must continue to rely on animal testing for evaluating the safety for humans of chemical compounds proposed for use in food-producing animals. Extensive evidence substantiates this view (Refs. 13, 49, and 50). Consequently, the use of animal tests is generally recognized and accepted by regulatory agencies as the principal basis for assessing potential risks from exposure to chemicals (Refs. 51, 52, and 53). This basis has been universally recognized and accepted by the courts (see e.g. *EDF* v. *EPA*, 510 F. 2d 1292 (D.C. Cir. 1975)). Moreover, the act does not distinguish beteen human carcinogens and compounds demonstrated to be carcinogenic in test animals. Instead, it assumes that an animal carcinogen presents an unacceptable risk of cancer in human beings. In this context, the issue of relevance to man of data from tests in animals must be refocused. In view of the strong policy in the general safety provisions of the act, which includes the Delaney clause, the primary regulatory objective must be to avoid falsely negative determinations of the carcinogenic potential of compounds under test in experimental animals. In this setting, the agency's only tenable regulatory posture is to select bioassay protocols that utilize test animal species/strains that are considered the best surrogates for man. The selection is based on available toxicologic and metabolic information.

Numerous terms are used to describe various proposed mechanisms of induction of chemical carcinogenesis, e.g., direct carcinogens, indirect carcinogens, promoter, initiators, cocarcinogens. The current knowledge of the mechanism of chemical induction of cance is generally not adequate to

permit these subtle distinctions. Further, the types of scientific studies necessary to identify precise modes of action for specific carcinogens are not yet refined to the point that they can be commonly applied (Ref. 54).

Moreover, the act does not distinguish between so-called "direct" and "indirect" carcinogens, and all types (assuming they are experimentally distinguishable) pose the same kinds of health risk to the public—namely, the risk of human cancer—that the act seeks to prevent. Therefore, the Commissioner concludes that there is generally no scientific basis for making regulatory distinctions among carcinogens.

The Commissioner agrees that short-term in vitro tests have a place in assessing the carcinogenicity of chemicals, as described in the preceding sections of this preamble, when they are intelligently applied and interpreted. However, the Commissioner does not agree that these tests can now substitute for long—term bioassays. The reasons for this conclusion were articulated by the expert committee of the National Cancer Institute on the use of these tests (Ref. 13).

At present, none of the short-term tests can be used to establish whether a compound will or will not be carcinogenic in humans or experimental animals. Positive results obtained in these systems suggest extensive testing of the agent in long-term animal bioassays, particularly if there are other reasons for testing. Negative results in a short-term test, however, do not establish the safety of the agent.

C. INTERPRETATION OF TEST DATA—IS THE COMPOUND A CARCINOGEN?

The majority of comments on the February 1977 notice requested greater specificity concerning classification of sponsored compounds as carcinogens, potential or suspect carcinogens, and noncarcinogens.

The objective of collecting and interpreting test data is to decide whether or not the compounds under test (the sponsored compound and any selected metabolites) are carcinogens. Within certain limits of confidence, statistical treatment of chemical carcinogenesis data can provide objective criteria for such determinations. To the question "Is the tested compound a test-animal carcinogen?" statistics can supply one of two types of answer:

(i) With "x" percent confidence (i.e., in "x" cases out of 100), "y" dose of the test compound will increase the carcinogenesis risk of test-animals over controls by no more than "s" and no less that "t"; or

(ii) With "x" percent confidence, "y" dose of the test compound will increase the carcinogenesis risk of test animals over controls by not more than "s."

An answer of the first type is possible only when the observed incidence of carcinogenesis in the test animals is significantly greater than that in the controls. When the observed incidence is the same for test and control animals, only an answer of the second type is possible.

A statistically significant increase in the incidence of carcinogenesis in one species or strain of test animals (i.e., an answer of the first type) is sufficient evidence to classify the test compound as a test-animal carcinogen. Because, for the purpose of these regulations, the act does not distinguish between human and animal carcinogens, a test compound as a test-animal carcinogen brings into play the requirements of the anticancer clause.

If the animal test data will permit only an answer of the type, the decision whether to classify the test compound as a test-animal carcinogen is more difficult. A negative test finding, as pointed out in some comments, can mean either that the test compound is not a test-animal carcinogen of that the bioassay protocol lacks a sufficient number of animals to discern an increase in the risk of carcinogenesis in the test animals. In those cases, a decision must be made whether to classify a tested compound as a noncarcinogen or to require further experimentation appropriate for resolving questions of safety. The Commissioner will conclude that a sponsored compound is not a carcinogen if the sponsored compound and each of the tested metabolites yields negative results. For purposes of these regulations, the Commissioner is proposing that the absence of a significant increase in tumor incidence in each of two different animal bioassays, conducted in accordance with good laboratory practices and designed according to principles referenced above, is (in the absence of other, positive data) sufficient evidence of noncarcinogenicity.

V. OPERATIONAL DEFINITION OF THE NO-RESIDUE REQUIREMENT

A. ALTERNATE OPERATIONAL DEFINITIONS

If it has been determined that a sponsored compound when admnistered to food-producing animals has the potential to contaminate edible tissue with residues whose consumption may pose a risk to human carcinogenesis, the agency cannot approve the sponsored compound unless it can be demonstrated that conditions of use can be established that ensure that the no-residue requirement of the act will be met. To establish those conditions of use and to provide a means for ascertaining whether these conditions are met in actual practice, some operational definition of "no residue" is necessary. Indeed, the act contemplates that the Commissioner will provide such operational definition, for there must be some criteria for prescribing or approving methods of examination for measuring residues.

The Commissioner has considered three basic alternative approaches to an operational definition of the phrase. Under one approach, the term "no residue" might be operationally defined as satisfied when the levels of residues fall below those that can be measured by available analytical methodology (alternative 1). A second approach would be to establish some low finite level (e.g., 1 part per billion) as a "practical zero" and to require assays that can reliably measure this zero, and to insist on the development of new assays if available assays are not adequate (alternative 2). Finally, "no residue" might be operationally defined on the basis of quantitative carcinogenicity testing of residues and the extrapolation of test data using one of a number of available procedures to arrive at levels that are safe in the total diet of test animals and that would, if they occurred, be considered safe in the total of man. Under this approach, the Commissioner would require assays that can reliably measure that safe level in edible tissues (alternative 3). For the reasons discussed in section V.B. below in this preamble, the Commissioner has concluded that alternative 3 should be adopted. The results of the carcinogenicity testing of the sponsord compound and any selected residues will be treated by the statistical procedures described in section V.

B. CHOICE OF AN OPERATIONAL DEFINITION

1. *Alternative one.* A number of assays might be development to measure the concentration of a chemical compound (i.e., residue) in an edible tissue, but for each there would be some level below which the compound under analysis could not be measured. (See section I.B. of this preamble.) Generally, different assays for the same chemical compound will have different, and sometimes vastly different, lowest limits of measurement. The no-residue requirement of the act could be translated an operational definition that is based solely on available analytical methodology and specifically on the lowest limit of measurement of an available assay. Thus, the degree of public risk associated with the use of a sponsored compound would become a function solely of the capability of available analytical technology.

The Commissioner concludes that this approach is unsound because it ignores all quantitative aspects of carcinogenicity testing. The carcinogenic potency of different chemicals varies widely. As used in this document, the

term "potency" refers to the dose required to produce a given rate of cancer. Disregard of "potency" in developing criteria for evaluating spnsored compounds would scientifically unsound, and would make no sense from the perspective of public health protection in accordance with the Delaney clause and the general safety provisions. Such disregard would produce situations in which residues of different compounds could present widely varying risks. The regulatory assays selected that way would not represent a consistent policy of protecting the human food supply from cancer risks. Indeed, the pattern of protection from one compound to another would be haphazard.

2. *Alternative two.* A second approach that the Commissioner considered was to establish a "practical zero" for the residues of all carcinogens. This approach would have one advantage over alternative one; it would provide a well-defined criterion for the lowest limit of measurement that any sponsor's assay would have to satisfy. This approach also would not, however, take into account differences in carcinogenic "potency" among various carcinogens. (See Table III.) Therefore, it is unacceptable for the same as alternative one. Unless the "practical zero" were set at the level appropriate for the most "potent" carcinogen, it would provide insufficient protection; but if it were set at that level, it might be unnecessarily stringent for carcinogens that produce a response that is of a lower magnitude. In sum, no one "practical zero" is appropriate for all carcinogens.

Moreover, under alternative two, the criterion for lowest limit of measurement probably would reflect consideration of what lowest level of measurement is "practical," given the state of the art analytical chemistry or biochemistry. In addition to failing to link the no-residue standard to any consideration of carcinogenic potency, this approach fails on the ground of practicality. The science and technology of analytical chemistry and biochemistry are continuously changing, and a lowest limit of measurement considered reasonable at one time would have to be discarded as unreasonable at a later time. Whenever a new and lower criterion for the limit of measurement would be established, the Commissioner would then presumably require that use of all compounds approved under the prior criterion be suspended until methods were developed to measure the residues at this lower level. Such a situation, in the Commissioner's judgment, would be both unreasonable and unmanageable.

On the other hand, to disregard advances in analytical chemistry and adhere to a previously established practical lowest level of reliable measurement with no public health rationale for doing so would be contrary to the statutory purpose and, ultimately, arbitrary and capricious.

A modification of the basic "practical zero" also has been suggested, i.e., that Congress intended FDA to adopt a practical zero set at the level of analytical technology at the time the various Delaney clauses were adopted. Under this theory for food additives, the practical zero would be set at the level of technology in 1958; the DES proviso would be governed by the level of technology in 1962; and new animal drugs, by the level in 1968. This uneven floor of technology is inappropriate not only for the reasons that make any "practical zero" level inappropriate, but also because it would be impossible for the agency to administer and has no basis in the policy or legislative history of the various amendments to the act.

3. *Alternative three.* A third approach to defining operationally the no-residue requirement is to establish a required lowest limit of measurement for each sponsored compound on the basis of data derived from measurements of the carcinogenic response resulting from various amounts of the compound itself or selected metabolites (Dose-response studies). A result of the increasing understanding of chemical carcinogenesis is that the question asked is no longer merely whether a substance is a carcinogen, but what is the amount required to produce a given incidence of cancer (Ref. 55). This concept of a dose-response relationship has long been used in medicine to determine safe and effective does of therapeutic agents. It is customarily used to describe the commonplace observation that in the majority of cases, different quantities of two differenct pharmacological agents are needed to elicit the same pharmacological effect (relative potency) (Ref. 56).

Both pharmacological effects and carcinogenic effects are biological effects, and there is no a priori reasons why the concept of relative potency should apply to the former but not to the latter. Carcinogenesis bioassays of increasing refinement conducted over the last 20 or so years have borne out this notion of relative potency for carcinogens. Thus, scientists ever more frequently speak of weak and strong carcinogens. In doing so, they express what is implied by the observation, for example, that dietary exposure to comparatively small amounts of 2-acetylamino fluorine causes bladder cancer in rodents at the same rate as does exposure to comparatively large dietary amounts of saccharin. Under this approach, relative carcinogenic potency is given specific consideration because actual chronic toxicity test data are used to determine the level of residues in edible tissue that an assay must be capable of measuring reliably. Thus, it permits a rational, uniform procedure for establishing the required lowest limit of measurement for assays and avoids the major deficiencies inherent in alternatives one and two. This approach directly carries out the statutory purpose of protecting the human food supply from residues that pose a carcinogenic risk to man.

Should new information develop on the dose-response relationship between the level of residues of a sponsored compound and the incidence of cancer, this approach would provide a practical basis for determining whether a new assay is required to establish compliance with the no-residue standard. Thus, this approach contributes to regulatory stability and predictability. Likewise, the Commissioner can provide the maximum public health protection based on quantitative carcinogenesis data. For these reasons, the Commissioner concludes that alternative three is the most appropriate means for implementing the statute and the most rational approach to developing an operational definition of "no residue."

By adopting this approach to implementing the no-residue standard, the Commissioner has assumed that: (i) The dose-response relationship between chemical compounds and carcinogenesis can be quantified, and (ii) a dietary level of a carcinogen can be identified at which no significant human risk of carcinogenesis would derive from consuming food containing residues below this level.

The dose-response relationships between compounds and carcinogenesis can be determined by testing in experimental animals, although the determinations are subject to known limitations inherent in every measuring device or system (Ref. 11). The second assumption, that residue levels representing no significant human risk of carcinogenesis can be assigned, protects the public from the potential and real dangers inherent in the interpretations of the "no-residue" standard of the act discussed as alternatives one and two. This second assumption and related issues are fully discussed in the next section of this preamble.

C. ANALYSIS OF ANIMAL CARCINOGENESIS DATA TO DEFINE OPERATIONALLY THE "NO RESIDUE" STANDARD OF THE ACT.

1. *Introduction.* The 1973 proposal included a modified version of the extrapolation procedure of Mantel and Bryan 1961 for use in defining the "no residue" standard for a sponsored compound (Refs. 57 and 58). The 1977 notice adopted a modified version of

the Mantel et al. 1975 procedure, which updated the 1961 procedure. The basic Mantel-Bryan procedure is one of several statistical techniques that allow estimation of the level, or dose, of a carcinogen that would lead to cancer rates in test animals well below detectable rates in practical experimentation. In normal experiments in which test animals are administered various levels (doses) of a suspected carcinogen, the observed responses (i.e., the percentage of test animals developing cancer if the compound is a carcinogen) usually range from about 5 percent to 95 percent. To observe responses at rates less than about 5 percent would require many test animals. Experiments designed to observe responses in the range of interest in establishing the "no residue" standard would require impossibly large populations of test animals. Therefore, the procedures of Mantel and Bryan and Mantel et al., as modified, were proposed respectively to be used in the statistical treatment of the dose-response data from actual experimentation to estimate the dose of the compound under test that would result in lifetime test-animal cancer rates no higher than a preselected rate.

Some operational zero must be defined in order for the "no residue" requirement of the act to be implemented. Regardless of the arguments for or against any particular procedure, the Commissioner maintains that the use of some procedure that quantitatively takes into account the carcinogenic potency of substances in test animals is far superior to any approach that fails to take that fact into account.

The modified Mantel-Bryan procedure described in the 1973 proposal was labeled excessively conservative (i.e., too protective of the public health) by some comments and recklessly liberal (i.e., insufficiently protective of the public health) by others. Those who considered the procedure too conservative objected to the proposed use of a series of conservative assumptions (shallow-slope dose-response relations, low acceptable level of risk, use of upper 99 percent confidence limits, etc.) and contended that any one of these assumptions alone could provide adequate public health protection. Further, these comments argued that the practical application of the procedyre had not been demonstrated, and suggested that it would prohibit the use of many valuable compounds.

Persons who considered the procedure too liberal objected to the proposed use of a lower confidence limit on the observed slope of the dose-response curve. They protested that the proposed statistical technique for extrapolating dose-response data obtained from animal tests seriously underestimated public risk. The technique provides a basis for establishing a dose level where there is no significant human risk of cancer, thereby establishing a criterion for a residue detection method. Specifically, the comments contended that if the true dose-response follows a logistic or linear distribution, extrapolation with the slope from a probit transformation would seriously underestimate public risk. Further, these comments argued that the probit transformation leads to a paradox because strong carcinogens are treated less conservatively than weak ones.

2. *Choice of the statistical procedure.* Most of the comments concerning the statistical procedure proposed in 1973 favored adoption of the Mantel-Bryan procedure without the modifications suggested in the proposal. A smaller number of comments contended that a linear extrapolation would be better than the Mantel-Bryan procedure and even fewer suggested the logistic or the angle distributions. Still other comments suggested that FDA require a comparative analysis of animal carcinogenesis data employing all alternative distributions, and that the smallest estimate of the "safe" level be used to define the "no-residue" standard for a compound. Finally, some comments stated that, although the logistic and angle distributions have been used in biological sciences, there is no indication that either one provides advantages over the probit (Mantel-Bryan) or the linear distribution, and that, therefore, neither is appropriate for regulatory purposes.

Some comments favoring the Mantel-Bryan procedure argued that it has a theoretical rationale that probably is relevant to the carcinogenic action of chemical agents. A similar argument was made by some of the comments favoring linear extrapolation. These comments also contended that linear extrapolation has the public health advantage of being the most conservative of all procedures.

In the period 1973 through 1977, the Commissioner extensively reviewed the known procedures that may be used to derive an operational definition of the no-residue standards of the act from animal carcinogenesis data. This review persuaded the Commissioner that the same scientific and technical limitations are common to all. Specifically, because the mechanism of chemical carcinogenesis is not sufficiently understood, none of the procedures has a fully adequate biological rationale. All require extrapolation of risk-dose relations from responses in the observable range to that segment of the dose-response curve where the responses are not observable. Matters are further complicated by the fact that the risk-dose relations assumed by the various procedures are practically indistinguishable in the observable range of risk (5 percent to 95 percent incidence) but diverge substantially in their projections of risks in the unobservable range.

In the 1977 notice, the Commissioner concluded that the comments failed to demonstrate that another procedure was superior to that of Mantel and Bryan and Mantel et al., and the Commissioner therefore adopted it with some modifications. Moreover, the Commissioner concluded that some aspects of the Mantel-Bryan procedure offered advantages over the other statistical procedures. It provided a means for pooling data from multiple experiments and from multiple dose levels within a single experiment, and thus permitted decisions based on the fullest use of available data. Further, the Mantel-Bryan procedure had a defined mechanism for handling the spontaneous tumor rate. To overcome certain limitations of the Mantel-Bryan procedure, the Commissioner adopted a number of modifications, which were discribed and discussed in the 1977 notice. The Commissioner also concluded that a review of the decision should be undertaken in 2 years and any appropriate modifications in the regulation initiated.

Since publication of the February 1977 notice, the Commissioner has received many additional comments on the statistical procedure chosen. Several suggested that the adopted Mantel-Bryan procedure is very complicated and requires a sophisticated computer program for handling and analyzing data and that such programs are not widely available. Also, a comment stated that the procedure uses a relatively untried mathematical theorem and applies it in a fashion for which it was never intended. Another comment contended that the Mantel-Bryan procedure is "disturbing" in that, for certain sets of data, it is possible that different answers will be produced by different starting points in the computer interation, i.e., there may be an infinite number of possible answers. A comment stated that neither Mantel paper was published in a recognized statistical journal, and, therefore, that the papers have not been subjected to proper peer review. Another comment argued that the procedure is based on unwarranted assumptions. Other comments suggested that the procedure is too lenient, and several suggested use of the linear procedure for extrapolation. Finally, another comment recommended the use of tne Hartley-Sielken procedure (Ref. 59) and contended that this procedure "has never been challenged."

In light of these comments, the Commissioner reexamined alternative statistical procedures for estimating

test animal exposure levels that correspond to specified levels of risk. None of the procedures suggested in the comments is known to be entirely compatible with current knowledge about chemical carcinogenesis. The procedure chosen must be that best supported by current science and also most protective of the public health. Of the three general procedures recommended by the comments or available in the literature (the curvilinear models, linear extrapolation and the Mantel and Bryan procedure (Refs. 57 through 63)), the Commissioner has now decided that for purposes of this regulation, linear extrapolation best meets the above criteria:

(1) Of the available procedures, the linear procedure is least likely to underestimate risk. That is, at the level of acceptable risk (1 in 1 million over a lifetime), the maximum permissible dose of residues calculated by use of the linear extrapolation is usually lower than that obtained by the use of the other procedures.

(2) Linear extrapolation does not require the use of complicated mathematical procedures and can be carried out without the aid of complex computer programs. The Commissioner now agrees with those comments suggesting that the Mantel-Bryan procedure is, for such reasons, unsatisfactory. The curvilinear model of Hartley and Sielken (1977) and Crump et al. (1977), like the Mantel-Bryan procedure, have many computational difficulties and require data from several dose levels.

(3) No arbitrary selection of slope is required to carry out linear extrapolation. For this reason, the Commissioner believes that it possesses an operational advantage over the Mantel-Bryan procedure; again, the Commissioner agrees with those comments that pointed out this difficulty in the previously proposed procedure.

(4) an approach to risk estimation recently proposed by Cornfield (Ref. 64) has been suggested to the Commissioner. Although Cornfield's approach may have merit, its assumptions and concepts have not yet been sufficiently scrutinized, evaluated, and accepted for the agency to adopt it at this time, as illustrated by the recent dicussion in *Science* (Ref. 64).

(5) Finally, the Commissioner has accepted the recommendations contained in a report issued by an expert scientific committee of the Department of Health, Education, and Welfare (Ref. 63) Linear extrapolation was proposed as the procedure of choice by the members of this committee.

For the above reasons the Commissioner now proposes to adopt linear extrapolation for regulating compounds subject to these regulations. The Commissioner recognizes that alternative procedures may have merit. Accordingly, comments are solicited on the property of those alternative procedures and what is believed to be their advantages over the proposed linear procedure. Of particular interest is the applicability of the curvilinear procedures to an interpretation of data on time-to-tumor observations.

3. *Time-to-tumor and other considerations.* Serveral comments contended that the 1973 proposal was deficient because it did not address the time-to-tumor aspects of chemical carcinogenesis. Some comments pointed out that Albert and Altshuler have developed preliminary statistical relationships between low levels of carcinogen exposure and time of tumor manifestation (Ref. 65). These authors maintain that characterization of carcinogenic potential and potency on the basis of incidence alone is not appropriate because it ignores the life-shortening aspects of carcinogenesis. A comment of the same type was received in 1977.

The Commissioner generally agrees with these comments. He recognizes that he must consider all manifestations of chemically induced carcinogenesis, including decreases in latency times (life-shortening effects). Accordingly, the Commissioner has reviewed recent scientific publications that attempt to address comprehensively all manifestations of chemical carcinogenesis (Refs. 54, 59, and 65). These publications offer generalized statistical techniques purportedly suitable for estimating all types of risks from experimental animal data. As expected, they are complex in concept and demanding in skills required for use. Without prejudice toward the technical and scientific merits of these generalized techniques, the Commissioner proposes that the linear technique be adopted in these regulations. In the Commissioner's view, this simple-to-use technique can be adopted to deal with all manifestations of chemical carcinogenesis even though it was not originally elaborated with life-shortening effects in mind.

Simplicity of use, however, is only one aspect of the procedure that must be considered. Other important aspects are technical and scientific merits or deficiencies. Therefore, the Commissioner invites those interested and knowledgeable in statistical techniques for risk estimation to consider and comment on the scientific and technical merits or deficiencies not only of the procedure proposed but those of the curvilinear procedures as well. The Commissioner will review comments on the time-to-tumor issue and will make any appropriate modifications in the procedure finally adopted.

One comment in 1973 stated that "effects produced at higher dose levels * * * are useful for delineating the mechanism of action, but for any material and adverse effect, some dose level exists for man or animal below which adverse effects will not appear." The comment analyzed in detail the deficiencies of all statistical extrapolations and stated that approaches are available to define a true carcinogenic no-effect level. It contended that it is more appropriate to determine a biologically insignificant level using a safety factor based on competent scientific judgment. In 1977, several comments reiterated the threshold issue but provided no supporting information or justification. Further, one comment has claimed that threshold levels have been established for 23 chemical carcinogens, although it provided no data or information to support this assertion.

The Commissioner disagrees with the contention that the classical toxicology concepts of the terms "thresholds" and "biologically insignificant levels" are generally applicable to carcinogenesis. There is substantial scientific controversy over whether these concepts apply to irreversible processes, such as the chemical induction of malignant neoplasia. The concepts of "threshold" and "biologically insignificant level" derive from short-term toxicity experiments. They have no established meaning with respect to biological processes that require long latent periods (up to 20 or 30 years) before the manifestation of lesions.

If it could be shown that there exists a threshold level for carcinogenic effects below which no member of the exposed human population would be at risk of developing cancer, and if a method were available to establish such a level for specific carcinogens, the Commissioner would seriously consider adopting such a level as the no-residue standard for this regulation. There is reason to believe, however, that the classic toxicological concepts of "thresholds" and "biologically insignificant levels" may not apply to carcinogenesis, and, further, that even if they do apply, there is no known method for establishing them in a manner that will provide the public health protection necessary.

It is true that "no effect" levels have been observed for some carcinogens in bioassays conducted in experimental animals. Such observed "no effect" levels should not, however, be mistaken for "thresholds" or for "biologically insignificant levels." There are several reasons for this conclusion.

In the first place, animal experiments are limited in their power to detect carcinogenic effects. Most such bioassays test approximately only 100 animals at each dose level. If no response is observed in 100 test animals, the upper 99 percent confidence limit

of the response is approximately 5 percent. Thus, there is a probability that a dose level producing "no observed effect" in this type of bioassay actually produces a response up to 5 percent; such a response (cancer incidence) can by no means be considered insignificant, even for the small test animal population, let alone for the entire human population of the United States. Of course, an observed "no effect" level in a carcinogenesis bioassay may indeed represent a "true no effect" level for the test animal population; there is, however, no way to ascertain which of these two possible interpretations of observed "no effect" levels is correct.

Even if it were assumed that a "no observed effect" level derived from a carcinogenesis bioassay represented a "biologically insignificant" level for the test animal population, it is unclear how knowledge of such a level would permit establishment of a threshold level for an exposed human population. Animal studies are performed under carefully controlled conditions that allow as little variation as possible in the environments of treated and control groups. The test animals have a uniform diet, are generally of the same age and state of health, and are otherwise living under uniform conditions. Further, the animals usually used in experimentation are genetically homogeneous.

By contrast, the human population exhibits a broad range of dietary habits, health status, age, occupational environment and genetic background; such factors are known to influence responses to toxic substances. For this reason, the human population is expected to exhibit a far broader range of susceptibilities to carcinogens than does the small and relatively homogenous test animal population for which "no effect" data may be available. Some segments of the human population may be less susceptible to the effects of a carcinogen, and some more susceptible, than the test animal group (Ref. 74). There is no information available that permits a quantitative determination of the relative susceptibilities of test animal and human populations. Therefore, it is not possible to devise a "safety factor" that can be applied to the animal "no effect" level (even assuming such a level were biologically insignificant for the test animal) to arrive at a level that can be considered safe for the entire human population. Moreover, if the animal "no effect" level is biologically significant for the test animal population (and, as has been shown this is not likely), the use of such a level to assign a safe level of human exposure, even after application of their safety factor, could lead to dangerously high levels of risk for humans.

Although the available information regarding the relative susceptibilities of test animal and human populations does not permit a quantitative determination of relative susceptibility, there are comparisons of a limited number of carcinogens (Refs. 66, 67, and 75). These comparisons only indicate that the lifetime cancer incidence induced by exposures in man can be approximated by the lifetime incidence induced by similar exposures in laboratory animals and that man may be no more susceptible than the most sensitive test animals species for which test data are available.

In addition to the variety of difficulties associated with methods for assigning threshold levels, there is considerable uncertainty whether such threshoulds actually exist. There is, for example, evidence that cancer can arise from a single transformed cell and that this transformation results from a single exposure and can occur long after the causative agent has been removed (Ref. 68).

The question of whether population thresholds exist for carcinogens is open for comment, and the Commissioner is willing to accept and take into consideration evidence that may develop on this issue. For the present, however, the Commissioner takes the position that there is no known method for establishing thresholds.

The Commissioner's view on this issue accords with that of an expert Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens contained in their Report to the Surgeon General, United States Public Health Service, April 22, 1970. The Report, which was published in full in "Chemicals and the Future of Man," Hearings before the Subcommittee on Executive Reorganization and Government Research of the Committee on Government Operations, United States Senate, April 6 and 7, 1971, contains the following conclusion:

> It is impossible to establish any absolutely safe level of exposure to a carcinogen for man. The concept of "toxicologically insignificant" levels (as advanced by the Food Protection Committee of the NAS/NRC in 1969), of dubious merit in any life science, has absolutely no validity in the field of carcinogenesis. Society must be willing to accept some finite risk as the price of using any carcinogenic material in whatever quantity. The best that science can do is to estimate the upper probable limit of that risk. For this reason, the concept of safe level for man, as applied to carcinogenic agents, should be replaced by that of a socially acceptable level of risk.

No information developed in the past 7 to 8 years warrants modification of this view.

Several comments opposing the proposal suggested that the agency should maintain flexibility and evaluate the approvability of sponsored compounds based on assessments of benfit and risk—in effect offering another approach to establishing the operational zero for carcinogenic residues. The Commissioner concludes, however, that an approach that contemplates considering the benefits of use of a sponsored compouund in defining the no-residue standard is incompatible with the anticancer provisions of the act.

It is the Commissioner's opinion, at least for new animal drugs, food additives, and color additives in animal feed, that it is improper to use risk/benefit considerations in making decisions about their safe use. The legislative history of the Food Additives Amendment of 1958 shows that the benefits of food additives are not to be considered in assessing whether they can be safely used. This position was strongly supported by the food industry. The industry feared that FDA would refuse to approve new, safe additives that provided only marginal benefits to the consumers or marginal improvements over additives already on the market (Ref. 69). Further, in that amendment Congress also added the flat proscription on the addition of animal carcinogens to the food supply. That action provides additional support for the position that (except for the very limited role assigned to the determination of functionality) risk is the only appropriate consideration in assessing safety under the food additive provisions of the act, which in large part governed the use of new animal drugs intended for use in food-producing animals from 1958 until the enactment of the Animal Drug Amendments in 1968.

As explained in Part I of this preamble, the legislative history of the Drug Amendments of 1962 shows that the DES proviso to the Delaney clause was added only to correct what Congress perceived to be an inequity in the regulatory system caused by FDA's application of the food additive provisions to the existing use of DES in cattle. But there is no basis for concluding that Congress by that action intended that an express risk/benefit consideration be added to the procedure for assessing the safety of substances intended for use in food-producing animals. Rather, Congress noted that the protection afforded the public would remain unchanged despite enactment of the proviso (see Part I.A.3 of this preamble).

The Animal Drug Amendments were enacted in 1968 to consolidate the various provisions of the act that were being used to regulate new animal drugs. The legislative history of that statute also contains no directive to FDA that the agency consider benefits in assessing the safety and approvabil-

ity of a new animal drug. In the absence of explicit Congressional direction on this point, FDA historically has considered it inappropriate to balance the risk of cancer that may be associated with the use of a sponsored compound (and assumed by one societal group) against the benefits that may be derived from the compound's use (and accruing to a different societal group). Recent case law in United States Courts of Appeals for the 5th and the District of Columbia Circuit has addressed different situations (see *American Petroleum Institute* v. *OSHA*, 581 F.2d 493 (5th Cir. 1978); Petition for cert. pending No. 1036 (U.S. 1979); *Agna Slide 'N' Dive Corp.* v. *CPSC*, 569 F. 2d 831 (5th Cir. 1978); *Environmental Defense Fund et al.* v. *Environmental Protection Agency*, No. 77-1091 (D.C. Cir. Nov. 3, 1978); and *Hercules Inc., et al.* v. *Environmental Protection Agency*, No. 77-1248 (D.C. Cir. Nov. 3, 1978).

4. *Expression of dose level.* Several comments received before the February notice addressed the adjustments the Commissioner had proposed to make in the "safe" level derived from the experimental animal data in order to establish an appropriate value for man. Some comments stated that adjustments for differences in food intake between experimental animals and man were inappropriate when dealing with carcinogens. The comments stated that such adjustments would assume erroneously that all toxic materials have the some mode of action on a body weight basis. They further suggested expressing the relationship in terms of concentration in the feed of the test animals and in the food of man when the diet in both cases in consumed adlibitum, not on an amount-per-body-weight basis. Other comments argued that the extrapolation of animal data to man should be based on body-surface-area ratios,.

The notice specified that carcinogenicity tests must be conducted with the test compound's concentration in the diet of the experimental animals held constant throughout the study. The safe or "acceptable" level derived from extrapolation of test animal data would be expressed as a concentration in the total diet (weight of compound/weight of total diet) of the animals and would be directly used as the acceptable level for the total diet of man. The Commissioner concluded that the arguments for conversion based on surface area ratios or on intake per unit of body weight have little basis. The comments provided no evidence that those concepts are applicable to low-dose chronic exposures. The concept of surface-area ratios is based on experience with short-term high-dose studies. Furthermore, measurements of surface area are crude. Finally, surface area and body weight will vary, as will food intake per day, throughout the chronic study, thus requiring constant adjustments of dose.

Until evidence is compiled demonstrating that there is a more appropriate means to extrapolate from experimental animal to man for chronic exposure and carcinogenic manifestation, the Commissioner will assume that the animal is the integrator throughout its lifetime of any observed response to a fixed concentration in the diet. The Commissioner has thus adopted the direct extrapolation approach (the safe level in parts per million, parts per billion, etc., of the diet of the experimental animals directly applied to the diet of man), which is appropriately conservative as well as the most practical of the approaches considered.

5. *Degree of data confidence.* The Commissioner disagrees with comments that characterized the proposal's requirement for 99 percent confidence intervals as another in a series of unnecessarily conservative assumptions. Confidence intervals characterize the quality of experimental measurement. The Commissioner maintains that a high degree of confidence should be demanded for decisions respecting carcinogens. The Commissioner therefore has adopted the 99 percent level of confidence, and the final regulations, reproposed herein, require that all calculations based on experimental observations be made from or with the 99 percent confidence limits.

6. *Slope used for extrapolation.* Because the Commissioner is proposing to adopt the linear model for risk estimation, comments on the slope used for the extrapolation are now irrelevant.

7. *Spontaneous tumor rates and data combination.* In the 1973 proposal the Commissioner recognized certain limiting features common to all extrapolation procedures, including that of Mantel and Bryan. These limitations concern the tumor incidence rate in the control groups of animal bioassays and the selection or combination of data from different experiments.

In response to comments, the Commissioner adopted in the February 1977 notice the procedure developed and utilized by Mantel et al. (1975) for handling spontaneous tumors. This procedure is an extension of the principles first articulated in the appendix to the 1961 Mantel paper and treats the rate of spontaneous tumors as an additional statistical parameter to be estimated from the data. The linear procedure in this proposal also treats spontaneous tumors in control animals as an additional statistical parameter to be estimated when two or more non-zero dose devels are utilized. When only one non-zero dose level is used for the linear extrapolation, an upper confidence limit on the increase in response of the dosed animals over the control animals is used. These methods of handling the data resolve some of the problems that arise when attempting to deal with spontaneous tumor rates.

Two comments in 1977 cautioned against the requirement for using the most "sensitive" test animals (i.e., the strain with the greatest tendency to develop tumors) as well as the "conservative" Mantel-Bryan procedure. They contended that these two requirements are incompatible because the high spontaneous tumor rate in the control animals reduces the number of animals that can manifest the effects of the chemical being tested.

The issue of sensitivity or susceptibility of the test animal species is relevant regardless of the statistical model selected for conducting the extrapolation. The commissioner does not intend to apply the term "sensitivity" or "susceptibility" in a way that is detrimental to the ability of the bioassay to detect carcinogenic potential, which has to be the overriding concern in selecting the test animal species.

In many instances, the male and female animals of the same strain may exhibit significantly different responses to a compound. Also, the responses of different strains and species may differ significantly. It is always desirable to make maximum use of available information by appropriately combining different data sets, but prudence must govern the process of selecting and combining data. Combining different data sets from the same or different experiments increases the number of animals used in the analysis and therefore increases the confidence in the results. Yet, in many instances, different data sets contain different types of information. Mantel et al. discuss the informational aspects of data combination for pooling data from different experiments and from different data sets in the same experiment. Although the Commissioner agreed in principle with most of their conclusions, it was nevertheless anticipated that situations would arise where the evidence in support of combining or not combining data would be equivocal. Therefore, the Commissioner concluded that the statistical and biological evaluation of data will determine which data sets, if any, will be appropriate for pooling. Where there are significant statistical and/or biological differences in the observed responses, only subsets of data representing statistically and biologically compatible bioassays will be combined for analysis.

Further comments on this segment of the February notice alleged that the agency's criteria for combining data are vague, arbitrary, and always unnecessarily conservative. A comment stated that FDA always combines the data to produce the highest risk regardless of the rationale for that combination. Other comments contended that cancer is a disease of old age. For this reason, it was argued, animal tests should be conducted in a way that reduces interference in the relevant observations caused by the high spontaneous tumor rates expected in animals of advanced age. It was also argued that, for the purpose of selecting data for a risk analysis, the agency should disregard all benign tumors occurring late in the test animals' lives.

There are many examples in which carcinogenic response to a chemical insult is limited to a segment of exposed human or animal populations, e.g., a single sex. It is only reasonable, therefore, that bioassay data be evaluated for the presence of such specific responses, and that the results of these analyses determine the ultimate manner of pooling data. These ultimate analyses are neither artibrary nor vague and are based on well-established scientific principles. Further, they do not always lead to the "most conservative" interpretation of the data; these analyses attempt to identify the data base that will result in the closest approximation of the true risk. In the Commissioner's opinion, this process is not regulatory "overkill" by any means; rather, an examination of the process shows that each decision in the process is independent and must be made on the merits of the data available. The proposed methods for combining data are, in each case, reasonable and well accepted, and the end result of the process is also reasonable because of the independent nature of the individual steps. For example, the regulation stipulates that the appearance of either benign or malignant tumors or both is evidence of carcinogenicity. As numerous experts have noted, both types of tumors will ordinarily be taken into account for the purpose of estimating risk as long as they are dose-related. Both types of tumors represent a carcinogenic threat, and neither can properly be ignored (Ref. 12).

The occurrence of tumors late in the life of test animals is also evidence of carcinogenicity as long as tumors are dose-related and occur at a greater rate in the treated than in the control animals. The Commissioner has no basis to ignore, as one 1977 comment suggested, the occurrence of benign tumors that occur late in life.

The Commissioner believes that the correlation between the type and rate of occurrence of tumors in the test animals and in man is poorly known and that to ignore benign tumors merely because they occur late in the lives of test animals would be imprudent.

8. *Level of risk.* The 1973 proposal suggested that an acceptable level of risk for test animals, and thus for man, could be 1 in 100 million over a lifetime. Many comments argued that this level of risk was unnecessarily conservative in light of the many other cumulative, conservative restrictions already in the proposed regulations. In the February notice the Commissioner concluded that the 1 in 100 million level of risk was unduly limiting without substantial compensation in terms of public health. Consequently, the notice established the maximum risk to be used in the Mantel-Bryan calculation as 1 in 1 million. The Commissioner explained the basis for selecting that level. Although additional comments on the level of risks were expressly requested, the Commissioner received only two comments on this issue. They contended that the level of risk selected was inconsistent with the congressional intent in enacting the proviso to the Delaney clause and was insufficiently protective of the public health.

Because Congress specified that the use of carcinogenic animal drugs and feed additives should leave "no residue" to be found (by methods prescribed by the Secretary) in edible tissue, it appears that Congress intended that the use of such animal drugs and feed additives not significantly increase the human risk of cancer from that use. It is also evident, however, that Congress intended to permit the use of carcinogenic animal drugs and feed additives if there would be no significant increase in the human risk of cancer from that use. Historically, safety decisions involving the use of chemicals have been made with the aid of numerical safety factors that do not consider the actual level of risk to the public. Observed no-effect levels from animal data are divided by an absolute safety factor to give a "safe" level for humans. For carcinogens, the Commissioner has concluded that it is necessary for the agency squarely to face the level of risk associated with a chemical compound's use before the agency will permit the use, and it is for that reason the Commissioner is proposing the statistical procedure for assessing risks prescribed in this document.

In the Commissioner's opinion, the acceptable risk level should (1) not significantly increase the human cancer risk and (2), subject to that constaint, be as high as possible in order to permit the use of carcinogenic animal drugs and food additives as decreed by Congress. For the following reasons the Commissioner believes that a risk level of 1 in 1 million over a lifetime meets these criteria better than does any other that would differ significantly from it:

(a) The risk level of 1 in 1 million is an increased risk over the entire lifetime of a human being.

(b) The upper 99-percent limit on the response data is used throughout the procedure, and the extrapolation procedure is conservative by nature. For these reasons, the maximum concentration of residues of carcinogenic concern that will go undetected in edible tissues is expected to increase the lifetime risk of excess cancer in humans by less than 1 in 1 million.

(c) This 1 in 1 million *lifetime* risk is expected only if the maximum concentration of residues potentially undetected in edible tissues is consumed every day over a lifetime. Because there is little likelihood that these residues will be so consumed by humans, the actual risk is likely to be lower than 1 in 1 million.

(d) The use of the procedures explained in the proposed regulations for deriving a concentration of residues that may go undetected in edible tissues rests on the assumption that the only risk to the exposed human population is that from residues of the sponsored compound. Other causes of disease or death are not considered. Because the population is constantly at risk from a wide range of factors, any increment of risk associated with residues subject to this proposed regulation is in comparison with other risks, likely to be vanishingly small.

(e) Several other prudent procedures apply to the derivation of the concentration of residues that will be permitted to go undetected (see section V.D. of this preamble below). For these and the above reasons the most likely human risk is expected to be less than 1 in 1 million.

(f) Once the level of risk is as low as 1 in 1 million, any further reduction in the level would not significantly increase human protection from cancer.

(g) An increase in the level of risk to 1 in 10,000 might significantly increase human risk. It is difficult to choose between 1 in 1 million and 1 in 10,000 but the agency chose the more conservative number in the general interest of protecting human health.

Furthermore, considerable discussion of the issue of acceptable level of risk has taken place recently (Refs. 55, 70, 71, 72, and 73), suggestions for the acceptable level of risk range from 1 in 20,000 per lifetime to 1 in 100 million. In addition to protecting the public health and satisfying the congressional directive, the Commissioner believes the selected level of risk should be consistent with acceptable levels of

risk for other materials that are considered safe, and should prevent any false sense of security in the calculations. After reviewing data on acceptable levels of risk and knowing the limitations on the procedures, the Commissioner has concluded that a level of risk of 1 in 1 million over a lifetime satisifies all of these criteria.

The Commissioner notes that for a few carcinogens, some limited comparisons have been made between risks estimated from animal experiments and those caluclated from human epidemiology studies (Ref. 66, 67, and 75). The tentative conclusion from these comparisons is that the lifetime cancer incidence induced by chronic exposures in man can be approximated by the lifetime incidence induced by similar exposures in laboratory animals. For this reason, the various conservative procedures and assumptions attached to the establishment of the permissible concentrations of potentially undetected carcinogenic residues should compensate for the possibility that for some carcinogens humans in general or some numerically significant groups of humans are more sensitive than test animals. Likewise, compensation must be made for the possibility of additive and multiplicative effects among the many carcinogens to which people are exposed daily. It is impossible to supply a quantitative estimate of the degree of compensation that results from the application of the various prudent procedures and assumptions. For these reasons the Commissioner has exercised caution by proposing an acceptable level of risk as low as 1 in 1 million.

In summary, the Commissioner has concluded that a risk level of 1 in 1 million over a lifetime imposes no additional risk of cancer to the public. A lower risk would not significantly increase the public health protection, but would probably proscribe the use of most animal drugs or feed additives. A risk level significantly higher than 1 in 1 million, for example 1 in 10,000, might present a significant additional risk of cancer to the public.

D. DERIVATION OF THE LEVEL OF TOTAL RESIDUES OF CARCINOGENIC CONCERN THAT CAN BE TAKEN AS SATISFYING THE NO-RESIDUE REQUIREMENT OF THE ACT.

As explained previously, a potential residue level corresponding to a lifetime risk of 1 in 1 million in test animals (i.e., the safe level derived from a statistical extrapolation procedure) can be considered the level that represents no significant carcinogenic burden in the total diet of man. This level was assigned the symbol "S_e" in the February 1977 notice, and expressed as a fraction in the total diet of the test animals, i.e., parts per billion, parts per trillion. The Commissioner concluded that it is the potential undetected residue level that is safe in the total diet of man.

In some cases, residues in addition to the sponsored compound itself will have been selected for carcinogenicity testing. In these instances, safe or acceptable levels will be derived for each of the compounds that has undergone testing. The compound exhibiting the lowest value for the safe level is the most potent carcinogen of those tested and poses the greatest potential carcinogenic threat among the residues. The Commissioner assumes that the smallest value of the safe levels of all the carcinogenic compounds tested represents the acceptable, total potential carcinogenic burden to man that may result from the administration of a sponsored compound to food-producing animals. This smallest value is assigned the symbol S_e. Because tested residues other than the one selected for S_e may have exhibited carcinogenic properties (although less potent) and still other, untested residues may represent carcinogenic risks, the sum of the levels of all of the residues must be less than S_e to ensure that any undetected residues do not present a significant risk of cancer to humans. Potential residues in the total human diet cannot exceed S_e if that diet is to bear no significant carcinogenic risk to man as a result of the residues. The only residues that can be excluded from the sum or residue levels are those that have been unambiguously shown to be noncarcinogenic in accordance with the principles described earlier.

One comment stated that the Commissioner failed to provide a mechanism to ensure that the total residue (S_e) will be accurately measured in edible tissues.

The comment has misunderstood the construct of the regulations. The S_e value is a projected acceptable total level of residue that is determined by calculations using bioassay (toxicology) data; it is not determined by totally individual analytical measurements. Therefore, the appropriate tasks with regard to safety are (1) determining the time when the total residues in edible tissue of target animals have depleted to S_e and below, and (2) selecting a suitable marker compound to monitor total residues. The determination of the expected time of the depletion of the total residues to S_e will be made in the second metabolism study, which is described in section VI below in this preamble. The second metabolism study will normally be conducted with radiotracer techniques that permit identification of a marker residue and target tissue. The regulatory assay will be used to monitor whether the total residue has depleted to S_e. The accuracy and precision of these techniques is well recognized and accepted.

E. CORRECTIONS FOR FOOD INTAKE

Several comments on the original proposal argued for, and others opposed, further adjustments based on patterns of food consumption. Some comments contended that the "safe" level of Mantel and Bryan in the animal diet should be directly applied as the upper allowable limit in man's diet and in any component food in the human diet. These comments argued that this limit should not be raised by considering the intermittency of consumption of particular foods or the proportion of the total diet represented by an individual food. They suggested that individuals who consume above average amounts of food would be exposed to above average, and thus possible harmful, levels of residues. Further, these comments contended that the act does not distinguish between the people who consume average diets and people who consume above average quantities of certain foods; the two groups are entitled to equal protection. They argued that adjustments for exposure frequency based on food consumption patterns assume that continuous long-term exposure to a carcinogen precedes the development of cancer.

Many other comments urged that adjustments be made based on the proportion of the specific food in the total diet and the frequency of exposure. These comments generally favored the use of food consumption data, so that the degree of conservatism would be more uniformly applied and would take into account the relationship of the particular food to the total diet.

The Commissioner disagreed with the contention that no adjustments should be made for factors of exposure. Section 512(d)(2)(A) of the act requires the Commissioner to consider the probable consumption of a drug and of any substance formed in or on food because of its use. All drugs, including carcinogens, are subject to the general safety provisions of the act. Consideration of the formation of chemical residues on food is necessary whether the drug is a carcinogen or a chemical toxicant of another type. There is no legal, scientific, or policy basis for concluding otherwise. The no-residue standard of the act has been defined as satisfied when the sum of the levels of all potential undetected residues of the sponsored compond (excluding only those that have been found to be noncarcinogenic) would not exceed S_e in the total diet of man. Because products derived from food-producing animals do not constitute the total human diet, it is appro-

priate that S_e be corrected for probable human consumption of specific tissues. The Commissioner agreed, however, that any adjustments must be conservative to assure that all segments of the population are protected.

Muscle tissue and eggs can be considered, conservatively, to each constitute one-third of the total daily human diet. Because milk can constitute the total daily diet of some individuals (e.g., infants), the Commissioner concluded that no adjustment for this commodity is appropriate. Adjustments for frequency of exposure for tissues other than muscle, milk, or eggs, (i.e., kidney, liver, etc.) will be considered when data are available that permit the Commissioner to conclude that the average daily intake of residues will not exceed S_e.

The February 1977 notice used the symbol "S_m" to represent the level of total residues of carcinogenic concern that can be operationally defined as satisfying the no-residue requirement of the act for specific tissues. The S_m value represents the level of residues that is acceptable for specific classes of edible products that constitute finite percentages of the total diet. Because milk may constitute the entire diet of an infant, the S_m value is its S_e value. But because muscle tissue constitutes one-third of the diet, the S_m value is 3 times the S_e value of the compound.

One comment on this section of the regulations said that the Commissioner was opening an avenue to permit as much as 20 times the S_e value in muscle tissue. This is emphatically not the case. The comment failed to recognize that the regulation establishes specific dietary conversion factors for muscle tissue, eggs, and milk (⅓, ⅓, 1, respectively), and conversions will be permitted for other tissues only when there are data to ensure that the S_e will not be exceeded in the total diet.

One comment raised a question about the quality of data used to establish the dietary factors for the major tissues, but the Commissioner concludes that the factors are correct. Although there are indications that the American diet has changed considerably in some areas in the past few years (e.g., the consumption of fabricated foods), there is no evidence that the consumption of muscle tissue, milk, and eggs, which serve as the basis for the basic dietary factors, has changed.

F. OTHER POSSIBLE ADJUSTMENTS

Several 1973 comments urged that the regulation not provide for adjustments for the degradation of residues in food under normal conditions of storage and cooking. Other suggested that this data should not be required, but should be taken into account when available. Still other comments expressed the fear that this data would be used to dilute the conservative intent of the regulation; they argued that the term "normal condition of storage and cooking" would be difficult to define, and it might reduce protection in situations where actual storage and food preparation practices did not approximate experimental conditions. Finally, some comments suggested that these studies be required only when there is reason to believe that the information would assist in protecting public health.

One comment on the February 1977 notice averred that the agency proposed to permit food with illegal residues to be marketed on the theory that violative levels of residues would "dissolve" before the food could be consumed.

The Commissioner agreed that the criteria appropriate to these studies were not defined, and he deleted the references to postslaughter residue degradation studies from the February 1977 notice. When there is reason to believe that storage conditions or food preparation methods might lead to the formation of potentially toxic residue products, however, the Commissioner will require appropriate special investigations. Petitioners are encouraged to explore the postslaughter stability of residues. Experience has shown that residue stability can be a complicating factor in studies for validating assays for dosed tissues. The Commissioner encourages research in this area; but until appropriate information can be reliably incorportated into food safety decisions, these data will not be used to liberalize the regulatory requirements.

G. OTHER POSSIBLE SAFETY FACTORS

Originally, the Commissioner proposed that the calculated does be modified to account conservatively for drug use patterns, e.g., the administration of the drug in the treatment of diseased animals. Comments stated that disease incidence does not occur randomly within a geographic area or within specific animal groups. Although a disease may have an overall incidence of only 10 percent, the affected group may be located in a single area. Therefore, the Commissioner was unable to conclude that evidence exists, or other safety factors are available, to permit the agency to calculate the effect of such drug usage, and this provision was deleted. No later comments have been received on this point.

VI. METABOLIC STUDY TO SELECT MARKER RESIDUE AND TARGET TISSUE

A. THE CONCEPT

Before the use of a sponsored compound can be approved, the Commissioner must determine that a practical and reliable assay is available to measure carcinogenic residues at the level which discriminates safe from unsafe food, i.e., the assay must be capable of determining when S_m is exceeded in each edible tissue. One approach to this problem would be to require assays that can be used to measure every residue in each of the various edible tissues. Because the number of residues in edible tissues and the number of tissues can sometimes be large, it is unlikely that such an approach would be practical. There is another far more practicable approach, which sacrifices no principle of safety. This alternative approach centers on the concepts of a marker residue and a target tissue.

A market residue is a residue whose level in a particular tissue is in a known relationship to the level of the total residue of carcinogenic concern in all edible tissues and which, therefore, can be taken as a measure of the total residue of interest in he target animal. Once a marker residue is selected and its quantitative relationship to the total residue is determined, it is possible to calculate a level, for purposes of these regulations, R_m, which is that level of the marker residue that must not be exceeded in a selected tissue (the target tissue) if the total residue of carcinogenic concern in the edible tissues of the target animal is not to exceed S_m. The marker residue can be the sponsored compound or any of its metabolites, or a combination of residues for which a common assay can be developed.

The target tissue is that tissue in which the absence of the marker residue at R_m or above can be taken as confirmation that the safe residue level, S_m, is not exceeded in any of the edible tissues. When a marker residue and a target tissue are selected, a practicable assay must be developed that can reliably measure the marker residue in the target tissue at levels at least as low as R_m, and conditions of use of the sponsored compound must be established that assure that, in practice, the potential marker residue level in the target tissue does not exceed R_m.

When it is determined, using an assay demonstrated to be capable of reliably measuring the marker residue in the target tissue at levels at least as low as R_m, that there is no such residue at levels at or above R_m, it can be concluded that the no-residue standard of the act has been satisfied for all edible tissues in the animal under ex-

amination. Conversely, if the marker residue is found in target tissue at levels equal to or greater than R_m, all edible tissues must be considered unsafe for human consumption.

B. APPLICATION: DATA COLLECTION AND CALCULATION OF R_m

1. *Marker residue.* Application of the concepts of marker residue and target tissue requires an experimental determination of the quantitative relationships of residues that might serve as marker residues (including any that have definitely been shown to be noncarcinogenic, because theoretically one of these might be selected as marker residue) to the total residue in each of the various edible tissues that might serve as target tissues. Further, because these relationships change with time, the levels of potential marker residues in the potential target tissues must be measured over time, and tissue concentration-time profiles must be constructed. These depletion profiles will be derived from measurements made in target animal tissues after cessation of exposure to the sponsored compound. Finally, because the results of carcinogenicity testing have been used to set limits for total potential undetected residues in each of the individual edible tissues, the depletion profiles must include measurements of the total residue in each potential target tissue to levels at least as low as the S_m appropriate to the tissue. Also, depletion profiles for one or more potential marker residues must be constructed and include measurements of levels of residues corresponding to the times when the total residue has reached S_m (Plates I and II set forth in proposed § 500.89).

Part III of this preamble describes the requirements for the study of the metabolic fate of a sponsored compound in target animals. Although the purpose of this earlier metabolic study is to provide information for selecting residues for carcinogenicity testing, the same principles and requirements are applicable here and must be followed in acquiring the information necessary to construct depletion profiles. However, to meet the depletion profile requirements prescribed by the regulations, a second metabolic study of the sponsored compound in the target animals may be necessary. This second and possibly more refined study may require using a larger number of animals. It will be necessary to determine the total number and the quantities of residues at several appropriate times, starting immediately after cessation of exposure and continuing until the residues in each of the potential target tissues have reached a level corresponding to a total residue level of the appropriate S_m for that tissue. If the initial metabolic study is done in a manner adequate to select a marker residue and a target tissue, of course, it need not be repeated.

Selection of a marker residue will be based on examination of depletion profiles. Generally, there will be a time at which the sum of the levels of the individual residues of carcinogenic concern will fall below the S_m appropriate to the tissue under examination. Residues that are potential markers will be present at a known concentration (R_m) at this same time (Plate I), and in a definite (although perhaps rapidly changing) quantitative relationship to the total residue (Plate II).

With the quantitative relationships established, it will be possible to select one of the residues as a marker. Ordinarily, the residue selected will have the following characteristics: (i) It will represent at least 10 percent, and usually more, of the total residue burden at the time the total residue was depleted to S_m; (ii) it will be stable, easily isolated and characterized, and susceptible to manipulation for assay development and implementation; and (iii) it will be undergoing relatively rapid change in concentration at the time the total residue burden is at or near S_m (i.e., a change in its concentration will be a sensitive indicator of the time when the total residue burden has depleted below S_m). Although other considerations may enter into the selection of a marker residue, these three will ordinarily be most important.

There may be instances in which no single residue can adequately fulfill the requirements a marker residue must meet. In such instances, it may be necessary to select some combination of residues which, taken together, can represent the total residue burden. It should be noted that a marker residue can be a compound which is not a carcinogen, but is an unambiguous indicator, in the manner already described, of the presence or absence of carcinogenic residues.

2. *Target tissue.* Selecting a target tissue requires a comparison of the depletion profiles for each of the edible tissues (Plate I set forth in proposed § 500.89). A target tissue will be selected on the basis of assurance that the absence of the market residue at or above R_m means that carcinogenic residues are absent from the tissue that requires the longest time to achieve its S_m, and thus that the entire animal is free of carcinogenic residues.

When a compound is to be used in milk- or egg-producing animals, milk and eggs will be target tissues in addition to one tissue selected as the target tissue to represent the depletion of residues in all of the edible carcass. In these cases, it may be necessary to select a marker residue for milk or eggs that is different from the marker residue selected for the target tissue representing the edible carcass.

3. *Calculation of R_m.* The R_m for a marker residue is the level of that marker residue which is present in the target tissue at the time, T_L, when the sum of the levels of the residues in the tissue that requires the longest time to achieve its S_m (excluding any residues that have definitely been shown to be noncarcinogenic) is equal to S_m for that tissue. The depletion profiles will be used to select R_m (Plate II set forth in proposed § 500.89).

For example, assume (i) that liver is the target tissue of animal drug, P, intended for use in cattle; (ii) that the only residues of P are the parent compound, P, and a metabolite, P_{SO_4}; (iii) that T_L is 3; (iv) that S_m for the sponsored compound is 29 parts per billion; and (v) that the following is a chart of the depletion profile of the drug.

Time	Total residue burden	P	P_1
0	100.00	75.0	25.0
1	65.4	41.6	23.8
2	42.0	25.3	17.3
3	29.0	15.0	14.0
4	21.0	9.0	12.0
5	15.0	5.0	10.0

In this case, before the drug can be approved for use, the petitioner must develop an assay that will satisfy the evaluation criteria in liver for either P at least as low as 15 parts per billion or P_1 at least as low as 14 parts per billion. Because P is depleting faster than P_1, when the total residue burden is 29 parts per billion, P may be the preferred compound to select as the market residue because it provides a more sensitive assessment of when the total residue burden reaches 29 parts per billion (S_m). Another example is provided in Plate II in proposed § 500.89.

Comments on the marker residue-target tissue segment of the regulations posed questions about the definition of terms and the implementation of procedures. One comment requested that the Commissioner add a table of definitions for the entire subpart, and it suggested that the agency coin a new term for the "marker residues." Another comment questioned whether the studies required to identify the marker residue and target tissue are truly "metabolism" studies. The February 1977 notice stated that the Commissioner would select the target tissue and marker residue, and one comment suggested that they be selected by the petitioner, who has a better knowledge of both the sponsored compound and of the availability of technology to develop assays for metabolities. Another comment questioned whether the agency is requesting sufficient information on edible

tissues to permit a determination of a marker residue or target tissue. It also questioned why the most slowly depleting tissue is not always the target tissue. It further requested that the target tissue concept be clarified when a target animal is used for milk or egg production.

The terms "marker residue" and "target tissue" are defined in proposed § 500.83, and their meanings will be codified by the final regulations. For clarity, a new section is added to define all new terms for the subpart. The term "metabolic study" has been used by FDA to describe the types of studies called for by the regulations for many years. The Commissioner disagrees that the term is inappropriate.

The Commissioner agrees that the petitioner for a sponsored compound has a role in selecting the marker residue and target tissue. Under current agency procedures, the selections are made with the opportunity for participation by the petitioner, and thus the petitioner's knowledge and proponent status are recognized. Because the agency must make the decision on whether the sponsored compound can be safely used, however, it must remain the ultimate decisionmaker.

The regulations require petitioners to determine the tissue depletion profiles for residues, and for a sponsored compound a considerable part of this information will already have been gathered by the initial metabolism study. (See section III of the preamble.) The Commissioner concludes that it is appropriate to select the target tissue from among tissues likely to become storage depots or to be involved in metabolism and excretion of the sponsored compound. Routinely examining other more specialized tissues in great detail will yield little additional useful information. Material balance calculations will be used as necessary to determine whether other tissues are potential storage depots and therefore may be target tissues.

The criteria for selecting the marker residue and target tissue are such that, when the marker residue concentration passes through its R_m in the target tissue, all other residues in the tissues, including the most slowly depleting tissues, will have passed through their R_m. Therefore, the most slowly depleting tissue need not be the target tissue.

Finally, the Commissioner explained in the February notice that for milk- and egg-producing animals, it is necessary to have a target tissue in addition to the milk or eggs. To clarify this matter, the Commissioner added this requirement to the regulations.

VII. Sponsored Compounds Affecting Pools of Carcinogenic or Potentially Carcinogenic Substances Endogenous To Target Animals

A. Applicability of No-Residue Requirement

The act requires that in making food safety decisions, the Commissioner take into account all substances formed in or on food by the administration of sponsored compounds to food-producing animals. It is well recognized that: (i) Several substances endogenous to food-producing animals are suspect or proven carcinogens (Ref. 64); (ii) in any given animal species or breed, the size of pools of such endogenous substances may vary widely and are affected by such factors as sex, age, lactation, state of estrus, pregnancy, and geographic location; and (iii) humans have had sustained exposure to such endogenous substances for centuries. Whether normal levels of human exposure to these substances are responsible for human carcinogenesis is unknown, but using drugs that can cause an increase in human exposure to these compounds has the potential of increasing the risk of human carcinogenesis. Under the act, therefore, the use of such drugs must be controlled.

In dealing with potentially carcinogenic endogenous compounds, the 1973 proposal declared that the intent of the no-residue requirement of the act is the maintenance of the normal human dietary content. Thus, the February 1977 notice required the determination of the effects of sponsored compounds on the normal background levels of potentially carcinogenic endogenous compounds. If a compound is found to increase these levels, conditions of use are to be established so that normal background levels are not exceeded in the animal when the animal is slaughtered. The notice also required development of practical assays for measuring levels of endogenous compounds.

Several comments on this segment of the 1973 proposal expressed concern over the meaning of the term "endogenous compounds" and questioned how these compounds are to be distinguished from "exogenous compounds." Others questioned whether the former term includes chemical derivatives (estradiol benzoate) of bona fide endogenous compounds (estradiol) or essential nutrients (some amino acids, minerals, vitamins). Comments also expressed doubt about the distinction between endogenous and exogenous compounds when the administered compound can be metabolized to residues of both classes. Some comments also argued that all externally administered compounds should be considered exogenous, as the true meaning of the term implies.

Other comments suggested that endogenous substances of interest be subjected to toxicological testing and tolerances be set if such substances are found to be not carcinogenic. Some doubted that available technology could meet the proposed requirements. They contended that the terms "normal conditions of use" and "normal background levels of endogenous compounds" would be either extremely difficult or impossible to define. While recognizing the difficulty of the task, the Commissioner concluded that administered compounds that increase the naturally occurring level of potentially carcinogenic endogenous compounds present special problems of control, which the proposed regulations had to address and resolve.

As the Commissioner explained in the February 1977 notice, an endogenous compound is any compound that is metabolically produced by and is present in untreated target animals. Any sponsored compound which, when administered to a target animal, is found to increase the normal background levels of a potentially carcinogenic endogenous compound is subject to these proposed regulations, regardless of how the increase is brought about. For instance, estradiol benzoate, which by the above definition clearly is not an endogenous compound, is metabolically converted to the endogenous compound estradiol and may thus cause an increase in normal background levels of that substance. Estradiol may itself be administered and possibly cause target animal pools of estradiol to increase above background. Finally, a sponsored compound may indirectly cause an increase in tissue levels of estradiol by affecting any number of hormonal regulatory systems in the target animals.

Although in each of the above-cited cases the cause of the increases in normal background levels of estradiol is different, the result is the same. And it is the result that must be monitored and controlled. It is thus of little use to distinguish between "endogenous" and "exogenous" administered compounds. Rather, it is useful only to distinguish between administered compounds that can cause changes in normal background levels of potentially carcinogenic endogenous compounds and those administered compounds that do not affect such levels.

Essential nutrients are not included in the definition of the classes of compounds that will be regulated by these proposed regulations. In a strict sense, essential nutrients are not endogenous. Although present in the tissues of animals and required for growth

and health, they are not produced by the animals and must be supplied from external sources. These features place essential nutrients in a distinct class of "required exogenous compounds," which must continue to be regulated in a unique manner. Determination of the allowable use of essential nutrients must reflect the target animals' nutritional requirements. When used according to label directions, supplements of essential nutrients that present carcinogenic risks should restore, but must not exceed, the essential nutrient levels found in natural foods adequately sustaining normal growth of healthy animals. Furthermore, the levels of such essential animal nutrients found in human food derived from animals with diets supplemented with essential nutrients must not exceed the levels in food derived from normal healthy animals fed a nutritionally adequate natural diet.

B. GENERAL PROCEDURES

If available information shows that a sponsored compound might affect pools of potentially carcinogenic endogenous substances above the level considered to be safe under the criteria of these proposed regulations, the petitioner would be required to investigate whether such effects occur under the conditions of the compound's proposed use.

The Commissioner proposes the following requirements: (i) Establishment of normal background levels (or "norm") of the endogenous compound of carcinogenic concern in the target animals; (ii) determination of the effects of the sponsored compound on the norm; (iii) establishment of safe conditions of use of the sponsored compound by demonstrating how the compound can be used in a way that ensures that the norm is restored in the target animals before slaughter; and (iv) development and validation of a practical assay to measure the endogenous compound at levels specified by the norm. The proposed regulations specify how each of these steps is to be accomplished.

C. SPECIFIC STEPS REQUIRED

The petitioner would first be required to determine experimentally the normal background levels, or norms, of the potentially carcinogenic endogenous compounds of concern in untreated target animals. A norm must be specific for the untreated target animals. The petitioner would provide the norm in the form of a cumulative frequency distribution of the observed levels of the endogenous compound. This curve must also include 99 percent confidence limits (Plate III appearing in proposed § 500.89).

The median and shape of the frequency distribution must be known so that shifts in the norm can be measured. For this reason, the assay used to determine a norm must yield values for the endogenous compound different from zero for at least two-thirds of the untreated target animals. This latter requirement is a compromise between the need to determine the frequency distribution with a high degree of reliability and at the same time to recognize the difficulties thay may be encountered in measuring levels at the lower end of the norm.

The petitioner would then determine the effects of the sponsored compound on the norm and provide data on the postexposure decay of any observed increases in the norm. The norm is considered restored when the distribution of values for the endogenous substance of concern observed in a group of treated animals is, with 99 percent confidence, the same as the norm.

The norm, as defined, takes into account those variables that affect background levels. The proposed regulations thus resolve the difficulties raised by 1973 comments suggesting that "normal background levels" would be difficult to define.

D. ENDOGENOUS MARKER RESIDUE CALCULATION OF R_m

If the norm of an endogenous substance of carcinogenic concern can be increased by the administration of a sponsored compound, the endogenous substance can become an endogenous marker residue, i.e., its presence above certain levels can be considered an indicator of potentially carcinogenic residues in food. Approval of the use of such a sponsored compound is contingent upon the petitioner's furnishing of data demonstrating that the norms are restored in the target animals before slaughter, and upon the availability of a practical assay that can reliably measure the endogenous marker residue in target animals. This regulatory assay must be capable of measuring the marker residue at the level, R_m, corresponding to the 33d percentile of the norm (Plate III set forth in proposed § 500.89).

The R_m for an endogenous marker residue derives from a conceptual approach entirely different from that used for the derivation of an R_m for an exogenous marker residue. To monitor shifts in the norm, the Commissioner must be able to measure the median and to determine the shape of the distribution. An assay capable of measuring the 33d percentile of the norm provides the analytical capability necessary to determine whether the norm has been shifted by administering the sponsored compound to the target animals because it permits measuring two-thirds of the points on the distribution curve. The same assay evaluation criteria apply to endogenous compounds as to other compounds covered by these proposed regulations.

Accordingly, the commissioner in the February 1977 notice revised the provisions which, as proposed, would have originally established the lowest limit of reliable measurement at the 99th percentile of the norm. As the comments noted, as assay that can measure only the upper 99th percentile would not be able to detect any shifts in the norm, which is its primary function. The proposed regulations require an assay capable of a lowest limit of reliable measurement of the 33d percentile of the norm, which will readily detect any shifts in the median or mean of the norm. Determination of compliance depends on a regulatory system that monitors shifts in the norms and not levels of endogenous substances in individual animals.

E. ALTERNATIVE PROCEDURE

Earlier comments contended that an alternative to the foregoing procedure should be available for regulating endogenous substances. It was suggested that a tolerance for an endogenous compound can be established at levels above the norm, provided that appropriate toxicity testing on the compound is carried out and a safe level can be established in accordance with sections IV through VI of this preamble and proposed §§ 500.84 through 50090.

Separate mechanisms with distinctly different rationales have been developed to measure compliance with the no-residue standard of the act for endogenous and exogenous compounds. As noted earlier, for exogenous compounds, the regulations would require development of an assay with a lowest limit of reliable measurement at or below the level needed to ensure that any undetected residues pose essentially no increased risk of cancer in the population. On the other hand, the method for measuring compliance with the no-residue standard for an endogenous substance is based on the norm.

In the absence of toxicology da'a of the type needed to determine a safe level for exogenous compounds, described in section V of this preamble, the Commissioner maintains that restoring the norm is the only way to ensure the absence of unaccepatble risks resulting from the use of compounds that may increase pools of potentially carcinogenic endogenous substances. If the toxicology data are available, however, and are suitable for extrapolation by the procedures described in section V of this preamble, the Commissioner will permit a shift in the norm equal to the incre-

ment shown to produce a lifetime cancer risk no greater than 1 in 1 million.

The 1977 notice announced that the Commissioner was receptive to suggestions for other alternative mechanisms of control. Two comments argued that the Commissioner has no authority to regulate increases in potentially carcinogenic endogenous substances that occur "indirectly" from the administration of the sponsored compound. They contended that the Commissioner can only regulate substances that derive directly from the sponsored compound, not from its use. The Commissioner rejects these comments, which are analogous to the earlier comments that the agency can regulate only a parent compound, not metabolites, under the Delaney clause. As explained in the February 1977 notice, the Commissioner is concerned about the use of compounds that may increase the pools of potentially dangerous endogenous substances that may be formed in or on food because of a sponsored compound's use. The general safety provisons of the act clearly cover all substances formed in or on food due to the use of a sponsored compound, and it is proper to consider excess levels of endogenous compounds of carcinogenic concern as such substances.

A comment requested that the Commissioner specify which potentially carcinogenic endogenous compounds are within the purview of this section. The Commissioner concludes that the proposed regulation covers all endogenous compounds that animal or human data show may present a carcinogenic risk.

Concerning the comment that all endogenous substances should be proscribed from use in animals, the Commissioner advises that there is no legal basis for their outright prohibition. Furthermore, the regulations prescribe procedures for use of these substances that ensure the same degree of safety as that required for the use of exogenous compounds.

Finally, a comment stated that the studies described in the February 1977 notice are costly, and it contended that, unless the data collected are considered proprietary, the requirement puts pioneers in the field at a disadvantage. The comment also requested that the Commissioner specify the studies required to define the norm and measure its restoration.

Under the current law, the Commissioner concludes that data on the norm are safety data required for every application and are proprietary data for new animal drugs. However, to reduce unnecessary testing, expenses to the regulated industry, and costs to the government, it is the agency's policy to encourage joint funding of tests.

The Commissioner believes it inappropriate to establish, as part of the regulations, detailed protocols for studies required to establish norms. However, the following example is offered as a guideline. To determine, with a high degree of confidence (99 percent), the characteristics of the distribution of the individual values that constitute the norm, the petitioner will ordinarily be required to examine a reasonable number of animals in each production class of target animals in which the sponsored compound is proposed for use, both treated and untreated. In each group, 450 to 500 animals will be sufficient to determine with 99 percent confidence:

(1) That the 99th percentile of the norm is less than the largest observed value; and

(2) That the cumulative frequency distributions of the observed levels of the endogenous compound in untreated target animals and in the treated target animals do not differ by more than .10 at any specific point.

To test whether the norm for the sample of untreated animals and the values for the sample of treated animals came from the same population, i.e., there was no effect due to treatment with the drug, the petitioner may use the Kolmogorov-Smirnov two-sample test. This test is concerned with the agreement between two cumulative frequency distributions. This test is sensitive to any type of difference in the distributions from which the two samples (treated and untreated) were taken, e.g., differences in location (mean, median, etc.), differences in variation, differences in skewness, etc.

The only assumptions required for this test are—

(1) That the samples are random samples;

(2) That the two samples are mutually independent; and

(3) That the samples are from a continuous population.

Specifically, the Kolmogorov-Smirnov test evaluates the probability of the maximum absolute difference that would occur between two cumulative distributions if they were obtained from the same population. For the details of conducting the test see Refs. 77 and 78. It must also be remembered that the above-described study may be conducted in lieu of chronic toxicity tests, and it can be conducted during the effectiveness studies. Thus the costs of developing and marketing an endogenous compound will be comparable to the corresponding costs for an exogenous compound.

VIII. Regulatory Assay: Evaluation Criteria and Approval Process

A. Introduction

The Commissioner can approve a sponsored compound for use in food-producing animals only if the intended use of the compound does not result in the accumulation of potentially carcinogenic residues in edible tissues and if an assay is available that can reliably measure such residues at and above the R_m. The assay must also be suitable for monitoring food from animals administered the compound to prevent food from reaching the marketplace if it is adulterated with potentially carcinogenic residues resulting from misuse of the compound.

Many comments in response to the 1973 notice contended that more explicit criteria and evaluation procedures should be specified.

The Commissioner agrees with these comments. Because the assays required by these proposed regulations are to be used for regulatory monitoring of residues of potential carcinogenic concern in food, rigorous criteria must be established for approval of these assays. Furthermore, the proposed assay must be subjected to an objective evaluation to determine whether it meets the criteria. Only then can there be assurance that an assay will provide a reliable and practical monitoring device to prevent violated residues in food. Most of the questions raised in the comments arose because the 1973 notice contained only a brief description of the assay evaluation criteria and procedures. Accordingly, the following discussion sets forth, as in the 1977 notice, the evaluation criteria and their bases.

Any assay used for regulatory purposes is characterized by a set of attributes that determine its quality: dependability, practicability, specificity, accuracy, and precision. These regulations specify objective criteria for these attributes. A proposed assay must be shown to meet these criteria during studies in a single laboratory and also in interlaboratory studies in government regulatory laboratories. The latter requirement is essential because the assays are to be used in Federal regulatory laboratories (FDA, USDA) and State laboratories, and the Commissioner must determine in advance that an assay will perform satisfactorily in more than one such laboratory. The proposed regulations specify that the interlaboratory validation study must be carried out in those laboratories (USDA and FDA) that will be using the method in surveillance and enforcement programs.

The steps in obtaining approval of an assay are—(1) assay development and study by the petitioner to deter-

mine whether the assay satisfies the acceptability criteria; (ii) FDA review of the petitioner's study to determine suitability of the assay for evaluation in interlaboratory study; and (iii) interlaboratory validation study, again with approval contingent upon satisfaction of acceptability criteria.

B. SOURCES OF DATA TO SUPPORT THE ASSAY

Data from studies of an assay using three types of samples are necessary to support approval. The petitioner must prepare and analyze samples of target tissue to which known and varying concentrations of marker residue, including R_m and concentrations above and below R_m, are added ("spiked" tissues). The petitioner must also compare responses obtained from assays using these tissues with responses obtained from assays of target tissues known to be free of marker residues (control tissues). In plotting observed instrumental response versus concentration of marker residue, i.e., in constructing the analytical curve from these data, as many samples as possible should be run, preferably by different analysts, because interlaboratory validation of the assay will eventually be required. The variability among different analysts can be determined at the developmental stage and adjustments made before the assay is submitted for FDA review.

Before submitting an assay to FDA for review, a sponsor should be satisfied that it meets all of the evaluation criteria and also that it is consistent with general principles of good analytical practice. Past experience shows that a petitioner's failure to follow good analytical practices during initial assay studies often results in interlaboratory failure even though the initial results may appear satisfactory during a paper review of the assay by FDA. A petitioner should assure that no results enter the construction of an analytical curve when it is known that the results were obtained using other than acceptable principles of analytical practice.

In addition to the spiked tissue tests, a petitioner must also submit data showing the applicability of the proposed assay to target tissues taken from target animals treated with the sponsored compound ("dosed" tissues). Validation of the assay requires dosed tissue samples that contain the marker residue at a level approximating R_m. The petitioner is required also to submit a standard analytical curve constructed by taking the marker residue of known purity at different concentrations, determining the response, and plotting the relationship.

C. SUBMISSION OF DATA

Agency resources for reviewing and validating assays are limited. The Commissioner therefore would establish in this proposal a precise format for submitting the data to support acceptance of an assay. It is a well-recognized principle, applied both by the courts and administrative agencies, that a standard format can be required for pleadings, requests for licenses, and other applications. This format may also designate special types of information that must be contained in the submission. Therefore, the agency would refuse to accept a petition or review an assay when the request for approval fails to conform to the format outlined below.

1. *Assay description and petitioner's evaluation.* The petitioner must provide a complete description of the assay to allow FDA to determine whether it is potentially acceptable. Because this threshold determination of acceptability will trigger an extensive interlaboratory validation procedure, the discussion must be sufficiently rigorous to minimize waste of agency resources. Therefore, the submission must discuss in detail—

(a) What equipment and reagents are necessary;

(b) How the assay is performed; and

(c) How the assay complies with the criteria of dependability, practicability, specificity, accuracy, and lowest limit of reliable measurement prescribed in proposed § 500.90(d) and discussed under section VIII. E. below in this preamble.

2. *Data.* The data and worksheets, including spectrograms, chromatograms, etc., from the spiked tissue, dosed tissue, and control tissue analyses and the external standard and quality control data are also necessary for the preliminary review of the assay to determine whether it actually complies with the evaluation criteria.

D. FDA REVIEW

The agency will conduct a paper review of a petitioner's submission to determine whether an assay complies with the acceptability criteria. These regulations generally alert potential petitioners to the applicable statutory standards and criteria, which should permit a petitioner to assess preliminarily the acceptability of an assay before filing a petition, and thereby reduce the agency's workload.

If on preliminary review an assay appears to comply with the evaluation criteria, it will then be subjected to the interlaboratory assay validation study to determine whether it is indeed a practicable and reliable regulatory tool. Should the initial review establish the assay fails to meet these criteria, the petition will be denied. A conclusion that an interlaboratory assay validation study should be initiated, however, in no way guarantees that a proposed assay will eventually be approved.

The assay criteria and attributes set out in the proposed regulations represent and amalgamation of statutory and scientific standards. Because a variety of terms are in use, the Commissioner is proposing to adopt and define the basic terms in the regulations in simple language for the sake of clarity. Accordingly, an assay must meet the following attributes and criteria for approval:

1. *Dependability.* Dependability is the likelihood that the proposed assay will not fail to yield a result because of uncontrollable features inherent in its design. Almost all assays will, on occasion, fail to yield any result. Often this failure occurs due to mishandling by the analyst, but sometimes failure may be the result of some aspect of the assay itself that may have been inadequately studied and defined or that cannot be controlled. For example, assays depends upon the availability of a standard against which measurements are compared. If the integrity of the standard depends on certian environmental factors (e.g., purity of the solvent in which it is maintained, temperature, light intensity, etc.) and these factors are understood, it may be possible to prevent assay failure. If this dependence is not know, however, the assay may fail and may fail often depending on the effect of the environmental factor of importance on stability of the standard. In this example, failure can mean a highly inaccurate result, assuming some fraction of the standard's intergrity is retained, or it can mean no result at all, assuming complete loss of integrity.

Assays used to monitor carcinogenic residues in food must be free of such uncontrollable features. Failure of a proposed assay to yield results during the petitioner's assay development studies or interlaboratory validation study can be a ground for refusing to accept the assay and for denying the underlying petition. Accordingly, the regulations require a petitioner to furnish information on, and provide an explanation of, runs of the assay that are begun, but never finished, during the analyses of samples used to construct the submitted analytical curve.

2. *Practicability.* Proposed § 500.90(d)(2) defines the practicability attribute as follows:

> The assay is considered practicable only if it is suitable for routine use in a government regulatory laboratory. The time required to complete the assay must be consistent with regulatory objectives, monitoring, compliance, etc. All supplies, equipment, reagents, standards, and other materials necessary to conduct the assay must be either commercially available, or readily available from the petitioner, on request. The Commission-

er will withdraw approval of any assay and initiate regulatory action against the sponsored compound if such a condition of the compound's approval is no longer satisfied.

The Commissioner has established criteria for practicability in terms that relate specifically to the nature of the laboratories in which the assay will be used, i.e., regulatory laboratories where the time and availability of equipment and reagents are critical factors in their ability to perform satisfactorily the mandate functions.

The inability to use an assay at a regulatory laboratory because a needed reagent is not readily available or because excessive time is requred to complete the assay presents potential risks to publish health and, therefore, precludes approval of the assay. Obviously, some assays will require some unique items, particularly reference standards. The Commissoner agrees with comments suggesting that, as long as a sponsor makes reference standards available to all persons having an interest, this requirement of the regulation will be met. A commitment to supply reference standards when they are not commercially available may be made a condition of the sponsored compound's approval, and failure to supply the governmental or other laboratories as required is a basis for withdrawing a compound's approval. The Commissioner concludes that an assay is not practical if it is dependent on the use of any other unique equipment or materials that are not commercially available.

3. *Specifity.* The regulations provide that, for an assay to be accepted, and observed response must be due to the compound that is being measured, and to that compound only. It is a fundamental part of the development of an assay to determine whether or not it possesses this important attribute. Among analytical chemists and biochemists, an "assay" that does not demonstrate this attribute is of little value; and indeed, in a regulatory setting, such an assay could be dangerously misleading. For this reason, the Commissioner has established rigorous specifications for this attribute.

In general terms, "specificity" refers to the uniqueness of the relationship between the observed effect (or response) and the applied stimulus (in this case the chemical under analysis). In analytical chemistry and biochemistry, the term "specificity" is commonly used to refer to the uniqueness of a response resulting from the application of a stimulus having specific characteristics; that is, the term has a qualitative dimension only in that it does not relate to either the quality of response or stimulus or to the nature of the relationship between response and stimulus. Both of the latter criteria, which might also be considered aspects of specificity, are central to good analytical practices. The regulations consider both the qualitative and quantitative aspects and groups them together under the general attribute of "specificity." The Commissioner's objective is to assure that, whatever the observed response, it is uniquely related to the marker residue both qualitatively and quantitatively.

The establishment of an analytical curve (not simple a standard curve, but one derived from actual measurements obtained on tissue samples containing known amounts of marker residue at different levels and from control samples) provides the means to detemine whether the responses produced by an assay are single-valued, as they must be if an assay is to be considered fully specific. Only assays that yield continuously increasing or decreasing analytical curves will satisfy the criterion of single-valuedness. The criterion of single-valuedness, or montonicity, must be established for the full range of possible contamination of residues, i.e., from zero residue levels up to levels of residues that will be present if no withdrawal period is observed.

The regulations require that the assay contain a sufficient number of independent measurements utilizing independent physicochemical principles to assure specificity (i.e., the identity of the marker residue must be confirmed). There are many ways in which specificity can be demonstrated experimentally. A petitioner may use highly sophisticated research tools to demonstrate that a proposed assay is specific in the ways discussed above. However, a regulatory analyst, using an approved assay, must have available some technique that can provide assurance that an observed response is due to the market residue. At present, although there are other possibilities, mass spectrometry is probably an ideal choice for acquiring the requisite specificity. Some determinations (e.g., those requiring enzymes) may have an inherent high specificity, but others have low specificity (e.g., gas, thin-layer, and liquid chromatography) and require other independent types of measurements to achive the requiste confirmation of identity. The requirement in the regulations that an assay contain a sufficient number of independent measurements negates the effect of a false positive measurement.

4. *Accuracy.* Assays yield measurements of concentration that are in some proportion to the true concentration of the compound being measured. The ratio of the measured to the true concentration of the compound, expressed as a percentage, is a measure of the assay's accuracy. The accuracy of an assay is determined from data collected from two types of studies.

One type of study must yield graphs of the observed concentrations of the marker residues, as determined by analysis, plotted against the corresponding levels of marker residue added to the analyzed target tissue. The plot is to be used to ascertain whether the assay meets the above-specified criteria.

The other type of study must measure the assay's recovery of marker residue from target tissue of target animals exposed to the sponsored compound. If target animals exposed to a radiolabeled sponored compound produce radiolabeled marker residue, it will always be possible to measure the proposed assay's recovery by directly comparing measurements obtained from the proposed assay and appropriate measurements of radioactivity. If it is not possible to have radiolabeled marker residue, the true concentration of marker residue in target tissue from exposed animals must be determined by exhaustive extraction of such tissues after appropriate standard treatments which hydrolytic enzymes.

The regulations prescribe specific accuracy criteria The average of observed responses must be between 60 and 110 percent of the true level of the marker residue when the lowest limit of reliable measurement, L_m, which is described in the next paragraph, is less than 100 parts per billion and between 80 and 100 percent of the true value if L_m is equal to or greater than 100 parts per billion. These criteria need not be satified throughout the full range of the analytical curve, but they must be satified in the range from L_m to three times L_m. These criteria are consonant with current good analytical practice.

5. *Lowest limit of reliable measurement* (L_m). To be accepted for regulatory purposes, an assay must be able to distinguish, with a high degree of confidence, target tissues that contain levels of the marker residue at or above R_m from target tissues that do not. This distinction must be reproducible and capable of supporting legal action when violative residues of the sponsored compound occur.

To provide the necessary degree of discrimination, the regulations require that the assay be capable of producing when the marker residue is present in target tissue at or above R_m a response that is, with 99 percent confidence, different from the response in nontreated (control) target tissue, i.e., the difference between the responses of control target tissue and target tissue containing the marker residue at or above R_m is, with 99 percent confidence, greater than zero.

The actual lowest limit of reliable measurement for the proposed assay is termed the "L_m", and it will be determined by reference to the analytical

curve of the proposed assay. The L_m will be the level of marker residue that gives a response above the expected blank value that is greater than, or equal to, 0.75 times the spread of the 99 percent confidence limits of a single assay response measured parallel to the observed assay response axis (see Plate IV in proposed § 500.90(d)(5)).

If the determined lowest limit of reliable measurement, L_m, of the proposed assay is equal to or less than the R_m, this criterion will be considered satisfied. This procedure takes into account the attribute of precision. Thus, an assay that satisfies this criterion will provide a reliable regulatory tool to enable the Commissioner to discriminate safe from unsafe food.

The Commissioner recognizes that the term "method sensitivity" is widely used to describe the lowest level of a compound under analysis that can be detected and measured with an analytical assay. Indeed, the original proposal used this term to describe what is now termed "the lowest limit of reliable measurement." However, there is some confusion surrounding the term "sensitivity." It derives in part from the fact that the term has been used in two senses: (1) As the lowest level of a compound that can be detected by an assay; and (2) as the lowest level of a compound that can be measured reliably by an assay. In fact, the correct meaning of the term "method sensitivity" is unrelated to a particular level of compound concentration, but rather relates to the ratio of change in instrument response to the change in compound concentration. The term "sensitivity" has therefore been dropped from this proposal. The Commissioner has adopted the term "lowest level of reliable measurement" because that term more accurately describes the attribute.

In response to comments urging that any "detected residue" should be subject to regulatory control, the Commissioner points out that it is an inherent characteristic of almost all analytical methods that componds can sometimes be detected at levels below the levels at which they can be reliably measured. More precisely, detection of a compound simply means that there is some instrument response above background levels that could be the compound of interest, but this response cannot be considered a reliable measurement or identification of the compound (Ref. 9). Since public protection is the goal, the Commissioner must be in a position to document conclusions based on analytical data, often in a court of law. A major aim of these proposed regulations is to assure that assays used to obtain such data can reliably measure residues. Hence, the Commissioner concludes that the discriminant for samples containing potentially violative exogenous marker residues must be the lowest limit of reliable measurement, L_m, of the approved assay.

Several comments on the 1977 notice stated that the definition of L_m and the procedures for determining L_m were incompletely specified. Most comments applauded the Commissioner's attempts to specify analytical attributes and agreed that the criteria were in accord with current good analytical practice. Several comments suggested that further specification of the interagency validation procedure might be desirable, and thus offered assistance if detailed guidelines were to be drafted in the future.

The Commissioner agrees with these comments and is proposing to define L_m in detail in the regulation as described above.

There was some confusion regarding the definition of "accuracy," and one comment stated that the regulations confused the terms "accuracy" and "recovery." The Commissioner agrees that in the February notice the term "accuracy" is used in a manner equivalent to what is normally termed "recovery." The term "accuracy," however, is more in line with analytical chemistry terminology, and the differences between accuracy and recovery occur only when dealing with absolute analytical methods, which will not be of concern here. For these reasons the Commissioner is proposing to retain the term "accuracy."

E. INTERLABORATORY VALIDATIONS OF ASSAY

Although FDA will review the assays for each sponsored compound, the actual regulatory field examination of foods of animal origin will be primarily performed by USDA under the Meat and Poultry Products Inspection Acts, and by the States under the Public Health Service Act. The Food and Drug Aministration performs a complementary regulatory function: Followup analytical and field investigations of violative residues to assemble evidence for use in regulatory actions.

The initial paper review by FDA of material in a petition permits the agency to make initial determination of the acceptability of an assay. Adequate protection of the public health, however, requires assurance that these assays will function in the government's regulatory laboratories. Therefore, these regulations also prescribe the procedure that will be used to assure that an assay is appropriate for use as as regulatory tool by government laboratories.

The Commissioner is proposing to require that three government laboratories (two FDA facilities and one USDA facility) independently validate an assay before it can be determined that use of a sponsored compound can be approved. This requirement is necessary because of the delicate nature of the assays, their importance in assuring that no residues of carcinogenic concern will occur in food of animal origin, and the practical limitations on the government's capacity to monitor food production and distribution. These three laboratories must study an assay sufficiently to assure that all criteria are met and that the petitioner has drawn correct conclusions in the submission about the assay's acceptability.

A comment on the 1977 notice suggested that FDA adopt the Association of Analytical Chemists' procedure for validating the assays. At this time, the Commissioner believes the AOAC process is inappropriate. It is very time consuming and permits testing in laboratories other those of FDA or USDA, where the assay will be used as a regulatory tool. Because of the delicate nature of the assays covered by these regulations and the time periods imposed for evaluating applications, the Commissioner declines to adopt the AOAC procedure. When the agency gains experience with the assays, however, the Commissioner will reconsider adopting in the regulations the AOAC assay validation process.

F. CONCLUSION

If an assay complies with the criteria described above and prescribed by the proposed regulations, and compliance can be verified under actual conditions of regulatory use (see section IX of this preamble), the Commissioner will approve the assay. A full description of the approved assay will be published in the FEDERAL REGISTER upon approval of the petition, in accordance with the provisos to the anticancer clauses and section 512(i) of the act.

IX. WITHDRAWAL PERIODS

A. INTHODUCTION

The regulations propose to define the withdrawal period for a sponsored compound as the time required, after cessation of target animal exposure to the sponsored compound, for the marker residue to deplete to L_m in the target tissue. The withdrawal period must also be compatible with actual conditions of livestock management and reasonably certain to be followed in practice. Because of the way in which the regulations define "marker residue," "target tissue," and "L_m," the use of a sponsored compound in accordance with the prescribed withdrawal period will assure that no carcinogenic residues of the compound will be present in human food derived from treated animals. At any point after cessation of exposure but before

the determined withdrawal period, treated animal tissues must be considered as containing residues of carcinogenic concern. Thus, the withdrawal period specifies the length of time after the last treatment with a sponsored compound in which animals must not be slaughtered for food and during which milk or eggs must be discarded.

Several comments on the 1973 proposal addressed the procedures for establishing post treatment withdrawal periods. Some contended that the requirement for tissue equilibration (no change in concentration of residues in the tissue with change in time) with residues in the experimental procedure for establishing withdrawal times was inappropriate for therapeutic drugs. Other comments suggested that the withdrawal periods be established to assure the absence of residues from edible tissues only, because they are the ones destined for human consumption. Some of these comments expressed concern about the practicality of applying confidence-interval techniques to establishing withdrawal periods, especially when dealing with large animals. Finally, one comment requested clarification on whether confidence limits or tolerance limits were to be used in setting withdrawal periods. The following paragraphs contain the Commissioner's response.

B. DATA TO SUPPORT WITHDRAWAL PERIODS

The depletion studies required by the proposed regulations to establish withdrawal periods must take into account the biological variability among animals and other vairables, e.g., assay variability, that may influence depletion times.

Residue depletion studies must be conducted under conditions of the sponsored compound's maximum proposed use. If a sponsor can demonstrate target tissue equilibration with the marker residue, however, a shorter period of administration than the maximum dose for the longest proposed conditions of use will be permitted. The conditions of the study must also simulate actual use conditions. The commissioner agrees that a compound intended for therapeutic use need only be administered according to the compound's maximum conditions of proposed use. The proposed regulatory assay must be used to measure the marker residue in the target tissue, including milk and eggs where appropriate, because it is this assay that will be used for regulatory monitoring.

All relevant data and evaluations must be submitted with the petition, along with a graphical presentation of the tissue depletion curve (concentration of marker residue in target tissue versus time).

The analysis of the data must include the estimated depletion curve, which in most instances may be adequately approximated by a first-order decay process. The statistical tolerance limit for the 99th percentile will be determined for the samples from individual target animals, and the time of intersection of this limit with the L_m value will be determined. The withdrawal period is the interval of time between the last administration of the compound and the time of intersection of this statistical tolerance limit on the observations and the L_m of the approved regulatory assay, plus an additional interval determined by rounding out this time interval to provide a practical withdrawal period compatible with animal management practices (Ref. 79).

For example, if the time of intersection of the statistical tolerance limit for the 99th percentile on the individual tissue determinations and the L_m for the marker residue is 39 hours, the withdrawal period (preslaughter interval) would be established as 2 days. In the case of milk samples, if the time of intersection were 63 hours, a withdrawal time of 72 hours (discard of six milkings) would be established.

The use of a compound will not be approved if the necessary withdrawal period is incompatible with animal management practices. For example, the use of a compound in lactating animals will not be approved if the required withdrawal time for milk exceeds 96 hours (4 days) because the management practices of milk production make observance of such discard times unlikely, or at least not reasonably certain, to be followed in practice.

When the marker residue is an endogenous compound, the withdrawal period is the time after cessation of administration of the sponsored compound required for the norm to be restored (see sections VII., C, D, and E above) and extended if necessary to be compatible with conditions of livestock management. The validated regulatory assay must be used to collect this information.

C. RATIONALE FOR USING THE STATISTICAL TOLERANCE LIMITS APPROACH

To establish that carcinogenic residues are absent from edible tissues of food-producing animals treated with the sponsored compound, the Commissioner must have information about the rate of residue depletion and the inherent metabolic variabilities among individual target animals.

The Commissioner is proposing to use statistical tolerance limits for this section to provide the degree of confidence (99 percent) necessary to ensure protection of the public health. Confidence limits, as used elsewhere in this regulation, estimate population parameters (e.g., 99 percent confidence limits will result in an interval that contains the true response rate 99 times out of 100). Statistical tolerance limits, however, are used to provide a specified degree of confidence that a specified portion of a population is below a given value (e.g., 99 percent confidence that, if the withdrawal period is followed, 99 percent of the target tissues will contain residue levels below L_m).

One comment on the February notice argued that withdrawal periods are unenforceable and contrary to the normal practices of the meat industry.

Section 512(d)(2)(D) of the act (21 U.S.C. 360b(d)(2)(D)) provides expressly that, in determining whether a compound is approvable, the Commissioner is to consider whether the conditions of use of a sponsored compound are reasonably certain to be followed in practice. Historically, safe conditions of use have included a preslaughter withdrawal period for many compounds intended for food-producing animals, and the compound's labeling requires that this period be discussed. In the Commissioner's opinion, withdrawal periods are being followed for most compounds, although some violation will always occur. However, one of the primary functions of this regulation is to improve the procedure for setting withdrawal periods and thereby provide FDA with stronger tools for enforcing compliance with withdrawal periods and for taking regulatory action if violative residues are detected.

Three comments raised questions about the use of the term "99 percent confidence interval." Another comment suggested that using the 99 percent confidence limits on the data in calculating the withdrawal period is too conservative and will result in unduly long withdrawal periods.

To clarify, the Commissioner has defined the term "99 percent confidence interval" in the proposed definition section. The Commissioner does not agree that the proposed approach is "too conservative." By using the statistical tolerance limit on the data, the Commissioner ensures with 99 percent confidence that in 100 sampled tissues there is no more than one violative residue when the labeled withdrawal period is followed. Minimizing the likelihood that a violative residue will occur is an important public health objective, and the Commissioner maintains that the procedures provided in these regulations (the use of a validated assay to collect residue data under proposed conditions of use; the use of statistical tolerance limits to establish withdrawal periods; and the use of good animal husbandry practice to aid

in determining whether withdrawal periods will actually be followed) provide the proper balance in setting a withdrawal period that ensures that (1) the food consumed, if the withdrawal period is followed, will be safe, (2) the withdrawal period is in accord with good animal husbandry practice and will be followed, and (3) violations can and will be detected.

Two comments raised questions about collecting data with the validated assay in the tissue depletion studies to determine the withdrawal period. Because assays are not validated until the final stages of a petition's review, the comments stated that it is impossible to collect data to establish a withdrawal period with the validated assay.

The Commissioner disagrees. For reasons already stated, the withdrawal period must be established with the assay for which approval is sought. Further, collecting the data by any method not proposed for validation imposes a repetitive administrative burden on the agency that is costly and unwarranted. When the data are collected with a different assay, the agency must first assess the quality of the data-collection assay and the appropriateness of the data submitted. Then it must attempt to compare the data-collection assay with the one proposed for validation. In the Commissioner's opinion this simply is an unacceptable waste of limited government resources; therefore, the Commissioner rejects any suggestion that the withdrawal period be established using an assay that is not submitted for validation.

A comment on withdrawal periods for endogenous substances contended that it is unnecessary to show when the norm is restored. The comment argued that merely showing that the norm is restored is adequate, regardless of when the restoration takes place. The Commissioner disagrees because the rate of the norm's restoration is an important consideration in setting the withdrawal period. It determines when food derived from treated target animals will be safe for human consumption. Only with such information can the necessary withdrawal periods be established.

Finally, two comments found unclear the statement that sponsors shall submit all raw data collected in determining withdrawal periods. They suggested that the regulation be reworded to require submission of all appropriate supporting data. The Commissioner agrees and intends to require submission only of all data that are relevant to determining withdrawal periods. Relevant data include, for example, descriptions of all assays on specific tissues, worksheets, and calculations, as well as daily calibration data (i.e., standard curves, spiked tissue, and background values).

X. Compliance

When a target tissue is examined with the approved assay and is found to contain the marker residue at or above its L_{ss}, the Commissioner will conclude that the carcass from which the target tissue was taken contains carcinogenic residues and, therefore, that the sponsored compound has been used in violation of the act.

When target animals are found to contain an endogenous marker residue at or above the 99th percentile of the norm (Plate III in proposed § 500.89(c)(1)(ii)), they will be designated as potentially violative. Because there is at least a 1-percent probability that untreated target animals will contain endogenous marker residue above the 99th percentile of the norm, further investigation will be necessary to determine whether the sponsored compound has been used in violation of the act. The function of this investigation will be to determine whether the potentially violative sample originated from target animals whose median level of the endogenous marker residue is greater than the median of the norm (and hence, the need for a regulatory assay having an L_{ss} at the 33d percentile of the norm). The proposed regulation also requires that, before regulatory action is begun, it must be determined whether or not the approved compound was used to treat the target animals under investigation.

Guarding against any shifts in the norms should allay all fears expressed in comments that monitoring only at the 99th percentile, as proposed, would not permit detection of any general increase in human exposure to potentially carcinogenic endogenous substances.

Food containing residues of any approved sponsored compound that has been used in accordance with the conditions of the compound's approval is specifically excluded from the adulteration provisions of section 402(a)1) of the act by sections 409(a), 512(k), and 706(a). Thus, administration of the sponsored compound according to the approved labeling is a defense to any criminal action that might arise for a violation of section 402(a)(1) of the act. However, within the meaning of section 402(a)(2) of the act, such food is adulterated if it contains a residue of the approved sponsored compound which is unsafe within the meaning of sections 409, 512, and 706. A residue is unsafe under those sections when it occurs in food at levels above those approved for use, and any residue found at levels equal to or above the L_{ss} is unapproved and therefore illegal. To establish that the residue is unsafe (an adulterant) within the meaning of sections 409 and 512 of the act, the agency must establish that the detected residue actually is a residue of the sponsored compound; and when the agency can prove this point, it has proved that the food is adulterated as a matter of law.

The proposed regulation requires each assay to meet specific criteria before the Commissioner will approve the sponsored compound or use, and an assay satisfying these criteria will permit the agency to discriminate between target tissue background responses and responses due to the marker residue. Levels of residues that are below the L_{ss} value cannot be distinguished from background with confidence, and the results of these findings are inadequate to support a regulatory action. On the other hand, when marker residues are detected and measured at or above L_{ss} with the approved regulatory assay, this finding will unquestionably support regulatory action since it constitutes evidence that the food is adulterated within the meaning of section 402(a)(2) of the act. (See *United States v. Ewing Bros. Co., Inc.*, 502 F.2d 715, 725–726 (7th Cir. 1974), cert. denied 420 U.S. 945 (1975).) Moreover, a finding of a violative residue will warrant further administrative action because it will constitute a prima facie case that the compound has not been used in accordance with its conditions of approval, and the agency will conduct a further investigation to determine what additional regulatory action, if any, is appropriate.

XI. Waiver of Requirements

The proposal would permit the Commissioner, in response to a petitioner's request or on the Commissioner's own initiative, to waive, in whole or in part, any of the foregoing requirements for the scientific evaluation of sponsored compounds that have the potential to contaminate human food with residues whose consumption could engender a human risk of carcinogenesis. It has long been settled that an agency may adopt a rule shown to be appropriate for the generality of instances and leave the correction of injustices to applications by those concerned (e.g., *National Nutritional Foods Ass'n v. Food and Drug Administration*, 504 F.2d 761, 784 (2d Cir. 1974) cert. denied 420 U.S. 946 (1975)). For these reasons, the Commissioner has expressly included the waiver provision. The Commissioner advises, however, that a waiver will be granted only in exceptional circumstances, and, as the regulation provides, the basis for any waiver must be documented.

XII. Implementations

The criteria set forth in the regulations are based on generally recognized scientific principles for testing and evaluating chemical compounds for potential carcinogenesis. Congress comtemplated that FDA would adhere to these principles when it enacted the Food Additives Amendment of 1958 and the Animal Drug Amendments of 1968 (21 U.S.C. 348 (b) and (c) and 360b (b) and (d)).

The 1973 proposal would have applied the regulatory requirements to all new applications (basic or supplemental) filed or approved after the effective date of the regulations. Prior approvals were to be dealt with on a class-by-class basis, and the classes, in order of decreasing priority, were known carcinogens, suspected carcinogens, and continuing through all compounds previously approved on the basis of zero tolerance. These were to be reviewed as part of the agency's general safety review for previously approved new animal drugs.

The February 1977 notice announced that the regulations would apply to all new animal drug applications, feed additive petitions, and appropriate color additive petitions, including appropriate supplemental applications, submitted after the effective date of the regulations. In addition, the regulations would apply to all pending petitions and applications unless the Commissioner determined that compliance with the act could be adequately assured by requiring completion of one or more of the required studies subsequent to approval.

Because some standards are needed for the day to day evaluation of petitions under sections 409 and 512, FDA has applied all the basic aspects of these proposed standards on a case-by-case basis for several years (e.g., diethylstilbestrol published in the Federal Register of November 26, 1976 (41 FR 52105) and the nitrofurans published in the Federal Register of May 13, 1976 (41 FR 19906) and August 17, 1976 (41 FR 34883)). It continues to apply them to compounds currently being evaluated for approval or subject to proposals to withdraw approval.

All previously approved applications for compounds will be reviewed as part of the cyclic review of the safety of marketed animal drugs, which will be described in detail in a separate forthcoming notice in the Federal Register. When the agency finds deficiencies in the data supporting a prior approval, it will issue either a Federal Register notice or a letter in accorance with section 512(e) of the act. The criteria of these regulations will be used to determine whether the data supporting applications are acceptable and adequate.

One comment argued that the final regulations, when promulgated, should apply only to all applications pending approval at that time. For previously approved compounds, the comment stated that the holders of the approvals should be required to submit data for at least a threshold assessment. For any compound found to require submission of additional data as set forth in the proposed regulations, the comment argued that the petitions for those compounds should immediately be suspended. Another comment, however, argued that the Commissioner lacks authority to apply the regulations to any previously approved compound without new evidence.

The Commissioner disagrees with both comments. The act expressly deals with these situations. It defines the new evidence that the Commissioner can consider in determining whether a previously approved compound is safe to include: "Tests by new methods, or tests by methods not deemed reasonably applicable when such application was approved, evaluated together with the evidence available * * * when the application was approved" (section 512(e)(1)(B)). The tests proposed in these regulations are necessary to show that a sponsored compound is safe under the act. For that reason, the absence of data satisfying the above criteria, in conjunction with the evidence already available about a compound, clearly can support the withdrawal of approval of an application. A reasonable implementation program is, of course, necessary to avoid chaos in the marketplace, permit an efficient application of the criteria, and provide the maximum public health protection. Proposed § 500.98 provides for such a plan.

XIII. Conclusion

The proposed regulations are designed to provide a comprehensive, systematic data collection procedure for evaluating the carcinogenic potential of chemical compounds intended for use in food-producing animals and to ensure that edible tissues derived from such animals are safe. The system is constructed with severable portions that can be modified or replaced as the capacity of science to resolve, or the need for resolving, the issues improves.

This regulation establishes a multistep procedure for evaluating the carcinogenic risk presented by a sponsored compound and criteria for the conduct of each step. In developing the steps and criteria, FDA applied high standards of scientific acceptability and public health protection. In the agency's view, each decision reflected in the regulations can be defended on that ground. The agency recognizes, however, that the totality of these decisions may impose a set of requirements that cannot feasibly be met by sponsors of compounds—for economic, technical, or other reasons. The agency, therefore, invites comments on whether the regulation imposes requirements that, as a totality, are unreasonable; and, if so, comments are invited on what specific provisions should be modified so that the requirements imposed by the modified regulation would be reasonable. Proposed modifications should be analyzed with respect to their impact on protection of the public health. No modification or set of modifications would be acceptable if its effect would be that the regulation would fail to provide satisfactory assurance that compounds approved for use pursuant to the regulation will not subject humans to any significant increase in carcinogenic risk.

The Commissioner has carefully considered the environmental effects of the regulations and, because this action will not significantly affect the quality of the human environment, has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk. (HFA-305), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857.

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Therefore, under the Federal Food, Drug, and Cosmetic Act (sections 402, 403, 409, 512, 701(a), 706, 52 Stat. 1046–1048 as amended, 1055, 72 Stat. 1785–1788 as amended, 74 Stat. 399–403 as amended, 82 Stat. 343–351 (21 U.S.C. 342, 343, 348, 360b, 371(a), 376)) and under authority delegated to him (21 CFR 5.1), the Commissioner proposes to amend Chapter I of Title 21 of the Code of Federal Regulations as follows:

PART 70—COLOR ADDITIVES

1. In Part 70, by amending § 70.50 by adding new paragraph (c), to read as follows:

§ 70.50 Application of the cancer clause of section 706 of the act.

* * * * *

(c) *Color additives for use as an ingredient of feed for animals that are raised for food production.* Color additives that are an ingredient of the feed for animals raised for food production must satisfy the requirments of subpart E of Part 500 of this chapter.

PART 500—GENERAL

2. In Part 500, by adding a new Subpart E, consisting of §§ 500.80 through 500.98, to read as follows:

Subpart E—Criteria and Procedures for Evaluating Assays for Carcinogenic Residues in Edible Products of Animals

Sec.

500.80 Chemical compounds used in food-producing animals: Procedures and criteria for determining acceptability of assays for carcinogenic residues in edible products.
500.83 Definitions.
500.84 Metabolic study in target animals to identify residues for chronic testing.
500.85 Criteria for test animal selection; comparative metabolic studies to aid in assessing the carcinogenicity of intractable residues.
500.87 Chronic testing.
500.89 Metabolic study to identify the marker residue and target tissue.
500.90 Evaluation and approval of a regulatory assay.
500.92 Withdrawal periods.
500.94 Publication of the approved regulatory assay.
500.95 Compliance.
500.96 Waiver of requirements.
500.98 Implementation.

AUTHORITY: Secs. 402, 403, 409, 512, 701(a), 706, 52 Stat. 1046–1048 as amended, 1055, 72 Stat. 1785–1788 as amended, 74 Stat. 399–403 as amended, 82 Stat. 343–351 (21 U.S.C. 342, 343, 348, 360b, 371(a), 376).

Subpart E—Criteria and Procedures for Evaluating Assays for Carcinogenic Residues in Edible Products of Animals

§ 500.80 Chemical compounds used in food-producing animals: Procedures and criteria for determining acceptability of assays for carcinogenic residues in edible products.

(a) *Scope of this subpart.* (1) The Food, Drug, and Cosmetic Act requires that compounds intended for use in food-producing animals be shown to be safe and that food produced from animals exposed to these compounds be shown to be safe for human consumption. The statute prohibits the use in food-producing animals of any compound found to induce cancer when ingested by human or animal unless it can be determined by methods of examination prescribed or approved by the Secretary (a function delegated to the Commissioner of Food and Drugs under § 5.1 of this chapter) that no residue of that compound will be found in the food produced from those animals under conditions of use reasonably certain to be followed in practice.

(2) Petitions for the approval of the use of a compound in food-producing animals must include adequate data for establishing the absence of residues of carcinogenic concern in the food produced from those animals.

(3) This subpart establishes the following: (i) The lowest limit of reliable measurement for the regulatory assay required for carcinogenic residues by sections 409(c)(3)(A), 512(d)(1)(H), and 706(b)(5)(B) and sections 409(b)(2)(D), 512(b)(7) and 706(b)(5)(A)(iv) of the act.

(ii) The procedures and criteria for evaluation and approval of such assays.

(iii) The procedures and criteria for establishing the premarketing withdrawal period for use of compounds likely to produce such residues.

(4) This subpart applies specifically to the use in food-producing animals and in their feed of compounds that have the potential to contaminate human food with residues whose consumption could present a human risk of cancer. The determination of this potential will be based on considerations of chemical, biochemical, physiological, and toxicological data derived from the scientific literature and from other sources available to the petitioner or to the Commissioner and on the proposed patterns of compound use. This subpart establishes a sequential process for the collection of other chemical, biochemical, physiological, and toxicological data pertinent to the

safety of the proposed use of the sponsored compound.

(5) This subpart does not apply to essential nutrients.

(b) *General approach.* (1) When the Commissioner determines that a sponsored compound has the potential to contaminate food from food-producing animals with residues (the sponsored compound, metabolites, or any other substances formed in or on food (e.g., endogenous substances) because of the compound's use) whose consumption could present a human risk of cancer, the following procedure for data collection and evaluation will apply:

(i) A metabolic study in the animals in which the sponsored compound is intended for use (target animals) designed to identify metabolites of concern and, when appropriate, to determine if normal levels of carcinogenic or potentially carcinogenic endogenous substances are affected.

(ii) Metabolic studies of the sponsored compound in different species of experimental animals designed to aid in selecting the appropriate species for chronic toxicity testing and in assessing the carcinogenicity of residues that cannot practicably be tested individually (intractable residues).

(iii) Chronic testing in test animal to assess the carcinogenic potential of residues of the sponsored compound, to furnish data suitable for statistical treament by the linear extrapolation procedure of Gross, M. A., O. G. Fitzhugh, and N. Mantel, "Evaluation of Safety of Food Additives," *Biometrics*, 26 (2): 181-194 (1970) and Hoel, D. G., et al., "Estimation of Risks of Irreversible, Delayed Toxicity," *Journal of Toxicology and Environmental Health*, 1:133-151 (1975)[1] (which are incorporated by reference), and to permit the no-residue requirement of the act to be operationally defined for purposes of establishing a lowest limit of reliable measurement for an assay to measure residues of the sponsored compound.

(iv) A detailed metabolic study of the sponsored compound in target animals designed to identify a specific residue and tissue to serve as indicators (marker residue and target tissue) to determine whether the no-residue requirement of act is satisfied.

(v) Development of a regulatory assay to measure the marker residue in the target tissue at and above the level operationally defined as satisfying the no-residue requirement of the act.

(vi) Establishment of the premarketing withdrawal period required for the safe use of the sponsored compound.

[1]Copies may be obtained from: Industry Information (HFV-226), Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(2) If, at any point in the sequential process of data collection set forth in paragraph (b)(1) of this section, the evaluation of the data satisfies the Commissioner that no human risk of carcinogenesis arises from the proposed use of the sponsored compound, the compound will be considered for approval under the general safety provisions of the act for risks other than cancer.

§ 500.83 Definitions.

The following definitions apply to this subpart:

(a) "Sponsored compound" means any drug or additive proposed for use, or used in, food-producing animals.

(b) "Target animals" means the production class of animals in which a sponsored compound is proposed or intended for use.

(c) "Sponsor" means the person proposing or holding an approval by the Food and Drug Administration for the use of a sponsored compound.

(d) "Threshold assessment" means the Food and Drug Administration's review of data and information available about a sponsored compound to determine whether the compound should be subject to regulation under this subpart as well as under the other general safety provisions of the Federal Food, Drug, and Cosmetic Act for risks other than cancer.

(e) "Total residue of the sponsored compound" means all compounds present in edible tissues of the target animal that result from the use of the sponsored compound, including the sponsored compound, its metabolites, and any other substances formed in or on food because of the sponsored compound's use.

(f) "Residue" means any single compound present among the total residue.

(g) "Residue of toxicological concern" means all compounds in the total residue minus any compounds shown to be safe.

(h) "Metabolic studies" means studies designed to identify the residues that occur in edible tissues when the sponsored compound is administered to target animals and to determine the depletion characteristics of the residues.

(i) "Intracable residues" means residues of the sponsored compond that, using the best available technology, cannot be obtained, by isolation, synthesis, etc., in sufficient amounts for carcinogenicity testing.

(j) "Comparative metabolism" means the study of the metabolism of a sponsored compound in different species/strains of test animals that are potential surrogates for man in chronic toxicity testing. Comparative metabolism studies will assist in assessing the toxicity testing. Comparative metabolism studies will assist in assessing the toxicity of intrectable residues and in selecting species/strains of test animals for bioassays of selected tractable residues.

(k) "S_s" means the residue level of a sponsored compound in a total test diet of animals that corresponds to a lifetime risk of cancer of 1 in 1 million in the test animals. For the purpose of this subpart, this S_s level in the test animal corresponds to a level in the total human diet that is assumed to represent a level of risk to humans of no more than 1 in 1 million over a lifetime.

(l) "S_m" means the level of total residues of carcinogenic concern for a specific edible tissue as determined by the formula in § 500.87(d).

(m) "Marker residue" means the selected residue whose level in a particular tissue is in a known relationship to the level of the total residue of carcinogenic concern in all edible tissues and that can be taken as a measure of the total residue of concern in the target animal.

(n) "Target tissue" means the tissue selected to monitor for residues in the target animal. The target tissue is selected so that the absence of marker residue at or above the required level of measurement (R_m) can be taken as confirmation that the safe, or acceptable, residue level (S_m) is not exceeded in any of the edible tissues of the target animal.

(o) "R_m" means the level of the marker residue(s) in the target tissue when the sum of the levels of the residues of toxicological concern is equal to S_m for the edible tissue requiring the longest time to deplete to its S_m.

(p) "Endogenous compound" means any compound that its metabolically produced by and is present in untreated target animals.

(q) "Essential nutrients" means compounds that are found in the tissues of untreated target animals and required for the animals' growth, and that must be supplied from external sources, e.g., essential amino acids.

(r) "Norm" means the normal background levels of an endogenous substance in untreated target animals, plotted as a cumulative frequency distribution of levels.

(s) "R_m for an endogenous marker residue" means the level of the endogenous marker residue that corresponds to the 33d percentile of the norm.

(t) "Spiked tissue samples" means samples of target tissue to which known amounts of marker residue have been added.

(u) "Control tissue samples" means samples of target tissue from untreated target animals.

(v) "Dosed tissue samples" means samples of target tissues from target

animals administered the sponsored compound.

(w) "L_m" means the level of marker residue in target tissue that gives a response greater than, or equal to, 0.75 times the spread of the 99 percent confidence bounds of a single assay response measured parallel to the observed assay response axis based on the analytical curve of the assay. (See Plate IV in § 500.90(d)(5).)

(x) "Assay" means the aggregate of all experimental procedures for measuring the presence of the marker residue of the sponsored compound in the target tissue of the target animals at or above the L_m. It includes the procedures for sample of instrument preparation. The assay must satisfy criteria set forth in § 500.90, and it will usually consist of multiple measurement procedures that utilize different physiocochemial principles, e.g., gas chromatography-mass spectrometry, to assure compliance with the regulatory requirements.

(y) "Withdrawal period" means the time required, after cessation of target animal exposure to the sponsored compound, for the marker residue to deplete to L_m in the target tissue.

(z) "Analytical curve" means the plot of the observed responses of the regulatory assay when analyzing "spiked" tissues compared to the amount of marker residue added to the "spiked" tissues.

(aa) "Ninety nine percent confidence interval" means an interval, determined by confidence limits, that is expected to contain the population parameter being estimated 99 times out of 100 times.

(bb) "Upper ninety nine percent confidence limit" means a value that is expected to be equal to or larger than the population parameter being estimated 99 times out of 100 times.

(cc) "Statistical tolerance limits" means upper and lower values between which it can be stated with a given level of confidence that a specified portion of the population will be included.

§ 500.84 Metabolic study in target animals to identify residues for chronic testing.

(a) A metabolic study, described in paragraph (b) of this section, shall be conducted in target animals to provide data on the physicochemical characteristics of residues, their relative proportions, their distribution among the various edible tissues (which include milk or eggs when applicable), and their retention and depletion in animal tissues.

(b) The metabolic target animal study shall satisfy the following minimum requirements:

(1) The metabolic study shall be conducted in target animals with the sponsored compound bearing appropriate radiolabels, unless other experimental methods permit measurement of total residues with accuracy and precision equivalent to radiolabel methods. Such labels shall assure that residues containing structural moieties of potenetial carcinogenic concern are detected and measured in edible tissues at levels as low as the best available technology will permit. Hypotheses about the sponsored compound's projected metabolic pathways may be used as a guide to experimentation, but they are not a substitute for actual experimentation.

(2) The dosing regimen shall be the maximum proposed use level and proposed duration of exposure to the sponsored compound. For a compound that is proposed for continuous or repeated use in target animals, administration for the metabolic study need continue only until tissue saturation has been demonstrated. If tissue saturation cannot be attained, residue equilibration or showing a stable metabolite profile will be adequate.

(3) The metabolic study shall be designed to yield the following information:

(i) The concentrations and total number of residues detacted in edible tissues of target animals immediately following cessation of exposure.

(ii) The concentrations and total number of residues detacted in edible tissues of target animals at a sufficient number of different time intervals, following the initial measurement, to determine the depletion trend of individual residues.

(iii) The physicochemical properties of the detected residues to identify compounds of potential carcinogenic concern.

(4) The results of the metabolic study shall be submitted in the form of a detailed report conforming to the standards required of scientific manuscripts submitted for publication in the journals of professional scientific societies, such as the American Chemical Society and the American Society of Biological Chemists. If addition, all raw data shall accompany and be referenced in the report.

(c) If the Commissioner determines that a sponsored compound has potential to contaminate food with residues whose consumption presents a human risk of cancer, the petitioner shall determine the carcinogenic potency of the sponsored compound and those residues that may be of public health concern due to chemical structure or persistence and concentration in edible tissues.

(d) Ordinarily, chronic testing of the sponsored compound and selected residues in experimental animals will be the preferred means of assessing carcinogenic potency.

(e) Reisidues in edible tissues of target animals that are intermediate metabolites in metabolic pathways that are reasonably expected to be similar in humans and the selected test animal species/strain need not be subjected to independent chronic toxicity testing. Testing the leading substrate in each metabolic pathway is sufficient. In the absence of information that the leading substrate is noncarcinogenic, tractable residues that are produced in the target animals but that are not produced in the test animal species/strain shall be subjected to independent chronic toxicity testing.

(f) Section 500.85 describes an alternative means of assessing the carcinogenic potency of residues whose isolation or synthesis in sufficient quantities for chronic testing proves to be beyond the practical limits of current chemical technology (intractable residues) by establishing additional criteria for selecting test animal species/strains used to conduct chronic toxicity testing of the sponsored compound.

§ 500.85 Criteria for test animal selection: Comparative metabolic studies to aid in assessing the carcinogenicity

(a) The primary criterion for selecting species or strains of test animals for chronic testing of both the sponsored compound and any metabolities selected in accordance with § 500.84 shall be the suitability of the species or strain as a model for man.

(b) If one or more intratable residues are also selected for chronic testing based upon the matabolic study in target animals, a secondary criterion shall be employed for selecting species or strains of animals for testing the sponsored compound. Metabolic studies of the sponsored compound in test animal species or strains determined to be suitable for chronic testing by the primary criterion shall be conducted to determine whether the intractabel residues present in the tissues of target animals are also produced in the test animals. Chronic testing of the sponsored compound in a species or strain of test animals in which the complement of residues produced is similar to the complement of residues produced in the tissues of the target animals is considered an appropriate method of assessing the carcinogenic potency of the intractable residues.

§ 500.87 Chronic testing.

(a) Chronic toxicity tests shall be conducted to assess the carcinogenic potential of the residues of the sponsored compound.

(1) The sponsored compound and any residues selected for chronic toxicity testing shall be subjected to oral, lifetime, dose-response studies in the test animal species or strains selected

in accordance with § 500.85. Each of these studies shall be designed to determine whether the test compound is carcinogenic. Protocols for these studies should be submitted to the Food and Drug Administration for review before commencing testing.

(2) On the basis of the results of these chronic toxicity studies and other available information, the Commissioner will determine whether any of the compounds tested is carcinogenic. If this evidence is equivocal, the compound will be regulated as a carcinogen until further testing resolves the remaining questions regarding carcinogenicity.

(b) When the Commissioner determines that a sponsored compound has the potential to increase the normal levels (pools) of carcinogenic and potentially carcinogenic substances endogenous to the target animals, the petitioner shall meet the requirements of § 500.89(c), (d), and (e) or (f).

(c) For each tested compound regulated as a carcinogen, the appropriate data from the chronic dose-response studies shall be analyzed according to procedures described by Gross, et al. and Hoel, et al. subject to the modifications and restrictions set forth in paragraph (c)(1) through (8) of this section. The purpose of this analysis is to interpret the "no residue" requirement of the act as it applies to the total residue of carcinogenic concern of the sponsored compound and thereby to determine the lowest level of reliable measurement required for a regulatory assay to be approved for the monitoring of the total residue.

(1) The administered dose of each test compound shall be expressed as a fraction of the total diet fed the test animal species/strains, e.g., parts per million, parts per billion.

(2) The permissible level, determined by the linear extrapolation model for each test compound in accordance with this section, shall be expressed as a fraction of the total diet fed the test animal species/strains. It shall be calculated using the 99 percent confidence limit of the observations for a maximum lifetime risk that is essentially zero but never expected to exceed 1 in 1 million.

(3) Data obtained from more than one dose level fed to groups of experimental animals of the same strain shall be combined as described by Gross, et al. and Hoel, et al. and are subject to the restrictions specified by these authors.

(4) Pooling data from various chronic tests using different animal sexes, species, or strains is permitted if it can be demonstrated that the protocols are of compatible design. If statistically significant biological differences in tumorigenic responses are observed between sexes or among species or strains of experiental animals, only subsets of data representing statistically and biologically compatible bioassays may be combined for analysis.

(5) All tumors, benign and/or malignant, shall be considered in the analysis.

(6) The number of animals at risk may be adjusted for competing risks unrelated to compound-induced carcinogenesis only when the data clearly support such an adjustment.

(7) When only the sponsored compound is subjected to chronic testing, the calculated "acceptable" level is to be designated as S_o. When more than one compound is subjected to chronic testing, the lowest of all calculated acceptable levels is to be designated S_o. S_o shall be expressed as the fraction of the diet fed the test animals, e.g., parts per million, parts per billion.

(8) The no-residue requirement of the act is considered satisfied when conditions and use of the compound, including any required withdrawal period, can be prescribed to assure that the sum of the levels of all potential residues of carcinogenic concern will not exceed S_o in the total diet of man, and a regulatory assay is available that is capable of reliably measuring such residues at and above that level. All residues of the sponsored compound are regulated as carcinogenic except those that have been shown to be noncarcinogenic.

(d) The S_o value represents the sum of all residues of carcinogenic concern that shall not be exceeded in the total diet of man. For individual edible tissues, the value that shall not be exceeded is to be designated S_m and calculated according to the following formula:

$$S_m = S_o/T$$

NOTE.—T is the fraction of the total daily diet of man represented by an individual edible tissue.

(1) The principal S_m calculations (defining T as noted in the formula above in paragraph (c) of this section) are as follows:

Edible tissue	T	S_m
Muscle	½	$3S_o$
Milk	1	S_o
Eggs	¼	$3S_o$

(2) Calculation of S_m for tissues consumed less frequently than muscle may take into consideration the frequency of consumption of those tissues if it can be clearly shown that S_o will not be exceeded in the total human diet.

§ 500.89 Metabolic study to identify the marker residue and target tissue.

(a) The petitioner shall conduct a study of the metabolic fate of the sponsored compound in target animals adequate to provide the data necessary for selecting a marker residue in target tissue.

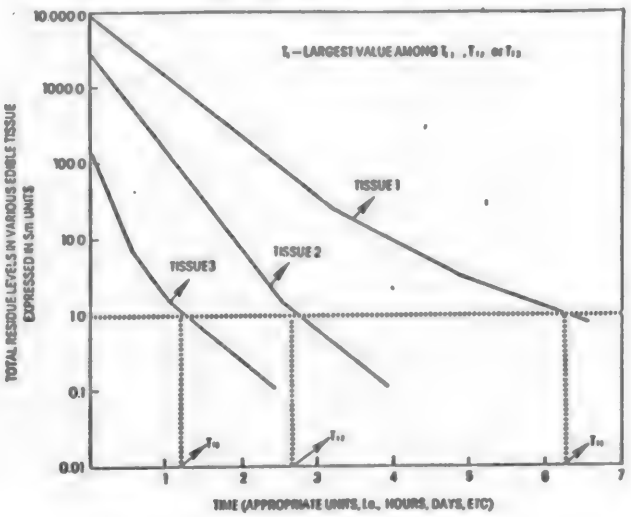
(1) The target tissue is that tissue in which measurement of the total residue burden of carcinogenic concern is a reliable measure of the total residue burden of carcinogenic concern in all edible tissues.

(2) The marker residue for the sponsored compound is that residue (the sponsored compound, any metabolite, or more than one of these) whose level in the target tissue is a reliable measure of the total burden of all residues of carcinogenic concern in all edible tissues.

(b) The metabolic study to establish the marker residue and target tissue shall comply with the requirements set forth in § 500.84(b) (2) and (4), with the following additional specifications:

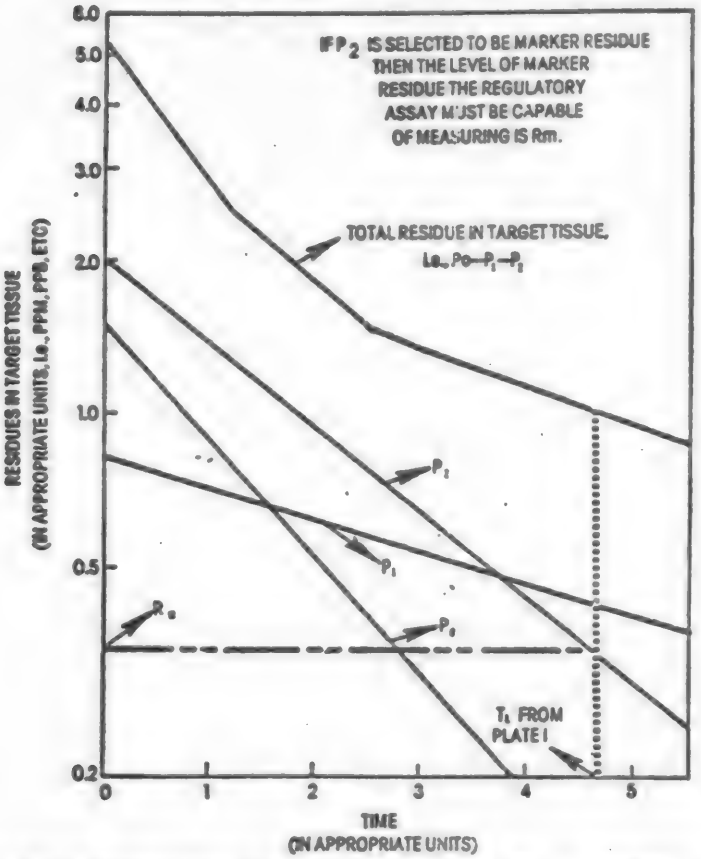
(1) For each edible tissue, the depletion profile of the total residue of carcinogenic concern shall be constructed and shall include measurements of levels at least as low as the S_m appropriate to the tissue under study, as set forth in Plate I as follows:

PLATE I. RESIDUE DEPLETION CURVES TO BE USED IN THE DETERMINATION OF MARKER RESIDUE AND TARGET TISSUE.



(2) Depletion profiles for one or more potential marker residues shall be constructed as set forth in Plate II as follows, and shall include measurements of levels corresponding to the time when the total residue level has reached S_m in the edible tissue requiring the longest time to deplete to S_m (T_1 of Plate I in paragraph (b)(1) of this section).

PLATE II. SELECTION OF MARKER RESIDUE AND ITS LEVEL Rm THAT MUST BE MEASURED BY THE REGULATORY ASSAY.



(3) If these specifications have been met by the metabolic study required by § 500.84(b), a second metabolic study need not be performed to satisfy the section.

(4) From these data, the Commissioner will select a marker residue and target tissue and will also designate the required level of marker residue, R_m (set forth in Plate II in paragraph (b)(2) of this section), that regulatory assays shall be capable of measuring in the target tissue. The selection of R_m will be such that the absence of the marker residue in the target tissue above R_m can be taken as confirmation that the total residue burden of carcinogenic concern does not exceed S_m in each of the various edible tissues and therefore that the total burden of carcinogenic concern in the human diet does not exceed S_m. When a compound is to be used in milk- or egg-producing animals, milk or eggs will be the target tissue in addition to one tissue selected to represent the depletion of residues in the edible carcass.

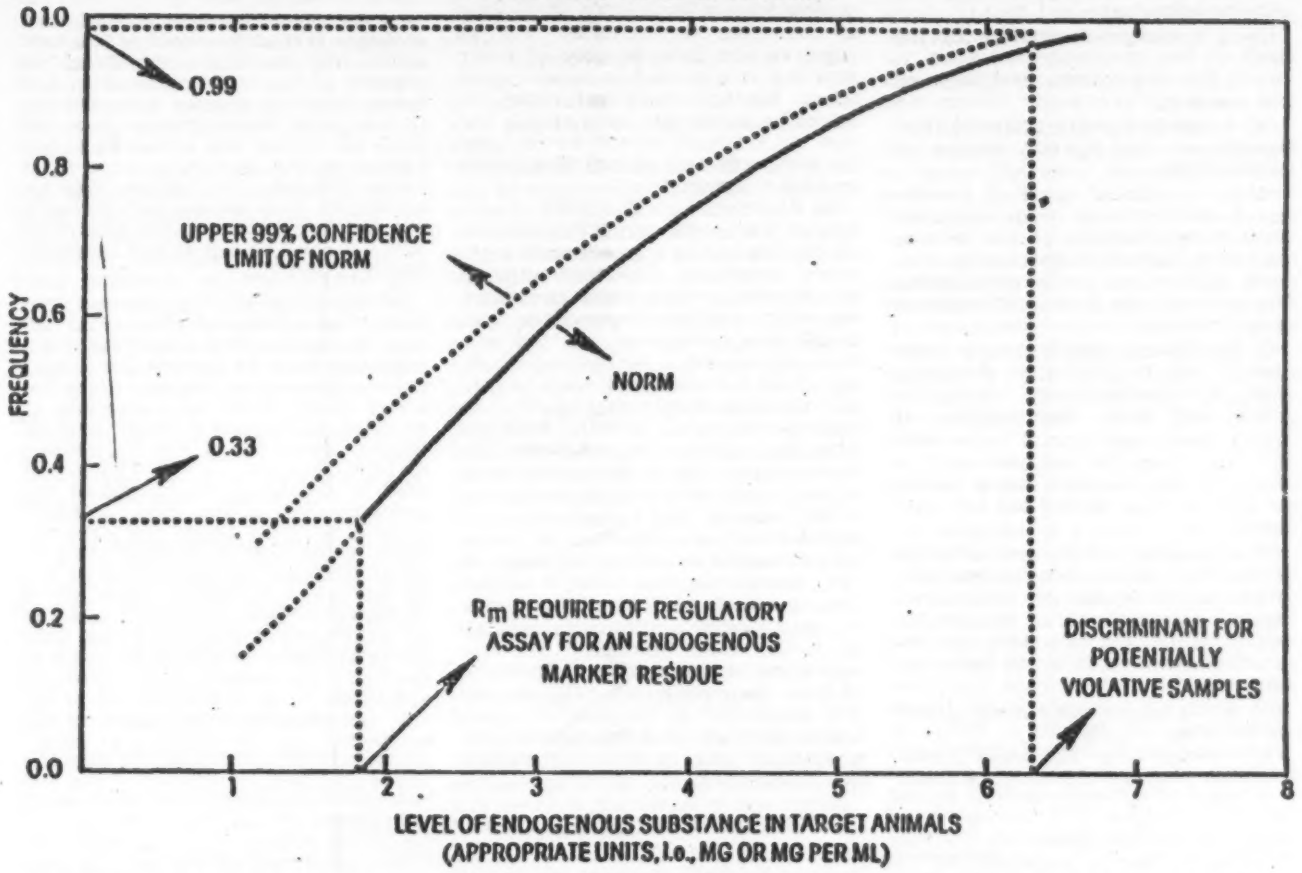
(c) When the Commissioner determines on the basis of available scientific information that a sponsored compound has the potential to increase the normal levels (pools) of potentially carcinogenic substances edogenous to target animals, the petitioner shall provide the following additional data:

(1) An experimental determination of the background levels (norm) of each of the potentially carcinogenic endogenous substances of concern in untreated target animals that are increased by administration of the sponsored compound.

(i) The norm shall be specific for the untreated target animals.

(ii) Each norm shall be submitted in the form of a graph of the cumulative frequency distribution versus the observed naturally occurring levels, including the upper 99 percent confidence limit set forth in Plate III as follows:

PLATE III. SAMPLE OF A NORM



(iii) An assay will be acceptable for the determination of a norm only if it yields values for the endogenous compound of interest greater than zero in at least two-thirds of the untreated target animals.

(2) Studies to measure the effect of the sponsored compound on the norm and the postexposure decay of any increase in the norm caused by administration of the sponsored compound. All data from these studies submitted in accordance with the requirements of § 500.84(b)(4).

(d) For a potentially carcinogenic endogenous compound whose norm is increased by the administration of a sponsored compound, the no-residue requirement of the act is considered satisified when the norm is restored.

(1) The norm is considered restored when, with 99 percent confidence, the cumulative frequency distributions of the observed levels of the endogenous compound in the untreated target animals and in the treated target animals do not differ by more than 0.1 at any specific point.

(2) The market residue is the affected endogenous substance.

(3) When the norm of more than one potentially carcinogenic endogenous compound is increased by administration of the sponsored compound, the market residue for all endogenous compounds of concern is that endogenous compound whose norm requires the longest time for restoration.

(e) For an endogenous compound selected to be a marker residue, the required level of measurement, R_m, for the regulatory assay is the level of that endogenous compound corresponding to the 33d percentile of the norm, set forth in Plate III in paragraph (c)(1)(ii) of this section.

(f) The Commissioner will permit a shift in the norm of a potentially carcinogenic endogenous compound if there are available toxicology data of the type specified by §§ 500.84, 500.85, 500.87, and 500.89 that permit estimation of a permissible level corresponding to a lifetime cancer risk increment no greater than 1 in 1 million. If the endogenous compound is also selected to be the marker residue, the required level of measurement, R_m, for the regulatory assay is the level of that endogenous compound corresponding to the 33d percentile of the norm set forth in Plate III in paragraph (c)(1)(ii) of this section.

§ 500.90 Evaluation and approval of a regulatory assay.

(a) Before an application is considered for approval, the petitioner shall submit for evaluation and validation a regulatory assay developed to monitor compliance with the no-residue requirement of the act. The regulatory assay shall reliably measure the marker residue in the target tissue at levels at least equal to and above R_m, as defined in § 500.89(b), (e), and (f). The criteria and procedures in paragraphs (b) through (g) of this section apply to the evaluation and approval of assays.

(b) The regulatory assay will be evaluated and validated using data collected from three types of samples:

(1) Samples containing various known concentrations of marker residue added to the target tissue, i.e., "spiked" tissue samples.

(2) Samples containing various levels of the marker residue obtained from target tissue at appropriate time intervals after the sponsored compound is administered in accordance with the proposed labeling, i.e., "dosed" tissue samples.

(3) Samples obtained from untreated target animals, i.e., "control" tissue samples.

(c) The petition for approval of the proposed regulatory assay shall contain the following:

(1) A complete description of the assay.

(2) A list of all necessary equipment and reagents.

(3) A standard curve prepared from samples of the marker residue of known purity.

(4) An analytical curve of the observed assay response compared to the tissue concentrations of the marker residue in spiked target tissue. The curve shall include the 99 percent confidence limits for individual predicted assay responses.

(5) All relevant data, including worksheets, calculations, any statistical analyses, spectrograms, chromatograms, etc., from the analyses of spiked, dosed, and control tissue samples, and from the analysis used in preparing the standard curve including data on runs started but not completed.

(6) A discussion of the data collected in the assay development process pertinent to the evaluation criteria set forth in paragraph (d) of this section explaining how the data show that the proposed assay conforms to those criteria.

(d) A regulatory assay shall satisfy the following criteria:

(1) *Dependability.* The assay is considered dependable if it does not result in an unreasonable number of failures due to unknown, uncontrollable, or random factors. Evaluation of the data to determine dependability will be based on the total number of assay runs that are started to provide data points for the analytical curve required by paragraph (c)(4) of this section. An explanation will be required for any assay run started that yields no final determination.

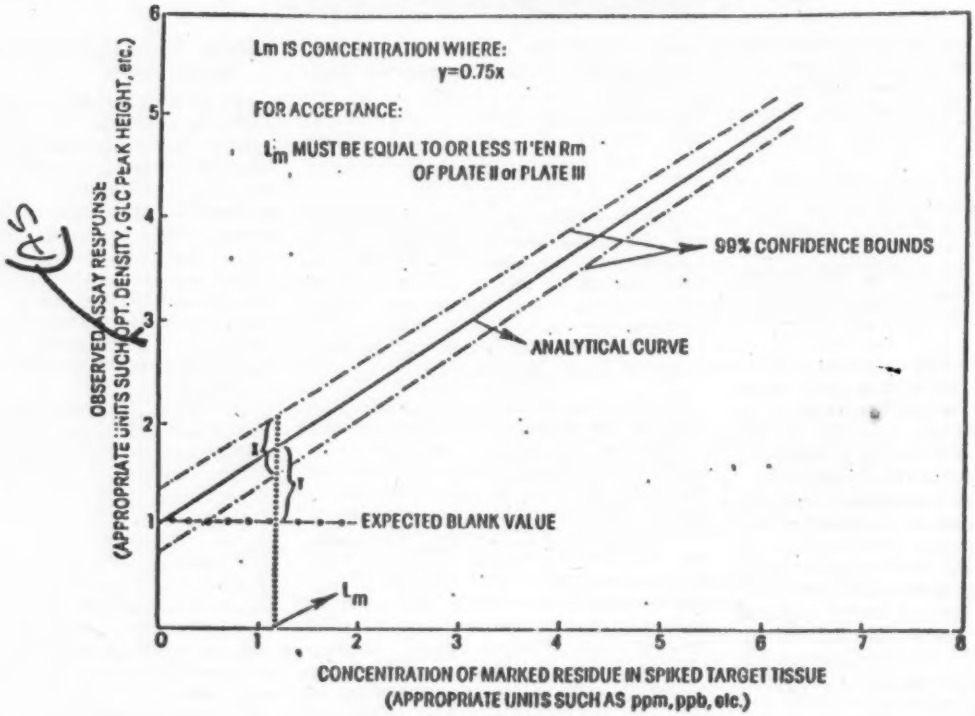
(2) *Practicability.* The assay is considered practicable only if it is suitable for routine use in a government regulatory laboratory. The time required to complete the assay shall be consistent with regulatory objectives, e.g., monitoring, compliance, etc. All supplies, equipment, reagents, standards, and other materials necessary to conduct the assay shall either be commercially available or readily available from the petitioner upon request. The Commissioner will withdraw approval of any assay and initiate regulatory action against the sponsored compound if such a condition of the compound's approval is no longer satisfied.

(3) *Specificity.* The assay is considered specific if the observed response is a smooth and continuously decreasing or increasing function of the concentration of the marker residue and of that compound only. The regulatory assay shall be composed of a sufficient number of independent measurements based on different biological, biochemical, or physicochemical principles to ensure that the identity of the marker residue is confirmed.

(4) *Accuracy.* The assay is considered accurate if the averages of the observed responses fall within 80 to 110 percent of the true value when the lowest level of reliable measurement (L_m) is equal to or greater than 100 parts per billion and within 60 to 110 percent of the true value when L_m is below 100 parts per billion. This requirement need not be met throughout the full range of the analytical curve; it shall be met in the range between L_m and $3L_m$.

(5) *Lowest limit of reliable measurement.* The regulatory assay is considered approvable if it can reliably discriminate with 99 percent confidence the marker residue response from the target tissue background response at or below the required lowest limit of reliable measurement, the R_m, defined in § 500.89(b), (e), or (f). The lowest limit of reliable measurement of the proposed assay is that level, L_m, which gives a response above the expected blank value that is greater than or equal to 0.75 times the spread of the 99 percent confidence limits on a single assay response measured paralle to the observed assay response axis (Plate IV below in this paragraph). If the L_m for the assay is at or below the applicable R_m of § 500.89(b), (e), or (f), the Commissioner will approve the compound for use only under conditions that will not result in residues above that level.

PLATE IV. ANALYTICAL CURVE OF A REGULATORY ASSAY



(e) The Commissioner will review and evaluate the data submitted in accordance with paragraphs (a), (b), and (c) of this section. If the assay satisfies the evaluation criteria of paragraph (d) of this section, it will then be subjected to the interlaboratory validation study described in paragraph (f) of this section.

(f) Two Food and Drug Administration laboratories and one U.S. Department of Agriculture laboratory will independently run a number of assays to ascertain whether the regulatory assay conforms to the criteria set forth in paragraph (d) of this section.

(1) The petitioner shall supply the validating laboratories with the number and amount of dosed and control tissue samples, requested by the Commissioner.

(2) The petitioner shall supply reagents, standards, supplies, and equipment to the validating laboratories, as requested by the Commissioner.

(g) The Commissioner will evaluate the data gathered from the study run by the three validating laboratories described in paragraph (f) of this section. The assay will be approved if it meets the criteria set forth in paragraph (d) of this section in each laboratory.

§ 500.92 Withdrawal periods.

(a) The withdrawal period is the time after cessation of administration of the sponsored compound necessary for the marker residue to deplete to the lowest level of reliable measurement (L_m) in the target tissue. This time is the interval required for the statistical tolerance limit for the 99th percentile of the marker residue concentration for individual animals to deplete to L_m. The time will be extended if necessary to be consistent with conditions of livestock management so that directions for use of the compound with respect to the withdrawal period will be reasonably certain to be followed in practice.

(b) The sponsor shall submit studies of the marker residue's depletion from the target tissue of animals dosed according to the maximum level of use proposed in the petition and maintained under field conditions. The validated regulatory assay shall be used to collect these data.

(1) The petitioner shall submit a plot of the concentration of marker residues in target tissue as a function of time (depletion curve) including the statistical tolerance limits for the 99th percentile of the expected marker residue concentrations for individual animals.

(2) All relevant data, including worksheets, calculations, and statistical analyses, shall be submitted along with a referenced discussion of the results.

(3) Use of the sponsored compound will be approved only if the available evidence demonstrates that the proposed conditions of use, including any withdrawal period, are reasonably certain to be followed in practice.

(c) When the marker residue is an endogenous compound, the withdrawal period will be the time required after cessation of administration of the sponsored compound for the norm to be restored, as described in § 500.89(d)(1). The time will be extended if necessary, but not reduced, to be compatible with conditions of livestock management so that the directions for use of the compound with respect to the withdrawal period will be reasonably certain to be followed in practice. The validated regulatory assay shall be used to collect data on the rate of restoration of the norm.

(1) The petitioner shall submit a series of curves that demonstrate the time required for restoration of the norm.

(2) All relevant data including worksheets, calculations, and statistical analyses shall be submitted along with a referenced discussion of the results.

(3) Approval of the petition for the sponsored compound will be granted only if the available evidence demonstrates that the proposed labeling is reasonably certain to be followed in practice.

§ 500.94 Publication of the approved regulatory assay.

The lowest level of reliable measurement (L_m), the complete regulatory assay for measuring the marker residue in the target tissue, and the analytical curve will be published in the FEDERAL REGISTER, in accordance with the provisions of sections 409(c)(3)(A), 512(d)(1)(H) and (i), and 706(b)(5)(B) of the act. For an endogenous marker residue, the norm will also be published.

§ 500.95 Compliance.

Compliance with the act will be determined as follows:

(a) When a target tissue is found to contain the marker residue at or above the lowest level of reliable measurement (L_m), the Commissioner will conclude (1) that the carcass from which the target tissue was taken is unsafe for human consumption; and (2) that the sponsored compound may have been used in violation of the act.

(b) When animals are found to contain an endogenous marker residue at or above the 99th percentile of the norm (Plate III under § 500.89(c)(1)(ii)), they will be designated as potentially violative. Before regulatory action will be initiated, and investigation will be undertaken. This investigation is to determine whether the potentially violative sample came from target animals administered the sponsored compound whose median level of the endogenous marker residue is greater than the median of the norm.

§ 500.96 Waiver of requirements.

In response to a petition or on the Commissioner's own initiative, the Commissioner may waive, in whole or in part, any of the requirements of this subpart for the scientific evaluation of sponsored compounds that have the potential to contaminate food with residues which, when consumed, could engender a human risk of cancer. A petition for this waiver may be filed by any person who would be adversely affected by the application of the requirements to a particular compound. The petition shall explain and document why some or all of the requirements are not reasonably applicable to the compound, and describe the alternative procedures that have been, or could be, followed to assure that use of the compound will not contaminate human food with residues whose consumption could engender a human risk of cancer and that an assay exists that satisfies the requirements of § 500.90(d)(1) through (5) and that is capable of measuring any residues that might occur when the compound was improperly used. Interagency validation of the assay will always be required. The petition shall set forth clearly the reasons why the alternative procedures will provide the basis for concluding that approval of the compound satisfies the requirements of the anticancer provisions of the act. If the Commissioner determines that waiver of any of the requirements of this subpart is appropriate, the Commissioner will state the

basis for the determination in the regulation approving marketing of the sponsored compound.

§ 500.98 Implementation.

(a) This subpart applies to all new animal drug applications, feed additive petitions, and relevant color additive petitions (i.e., applications and petitions concerning any compound intended for use in food-producing animals) submitted to the Food and Drug Administration, including relevant supplemental applications and amendments to petitions, and to all these applications or petitions on file with the agency. If the Commissioner determines that consumer protection can be adequately ensured by imposing the requirements under paragraph (b) of this section, the Commissioner will do so.

(b) This subpart also applies to the following compounds already approved:

(1) Those compounds that the Commissoner determines, on the basis of available information, have been shown to induce cancer when ingested by man or animals.

(2) Those compounds that the Commissioner determines may induce cancer when ingested by man or animals, i.e., suspect carcinogens.

(3) Any compound for which the Commissioner concludes sufficient information has not been provided to determine whether residues of the sponsored compound present a risk of cancer to man.

(c) Any compound already approved, to which the Commissioner determines the anticancer provisions of the act apply, or for which additional data are required for such a determination, will be the subject of a notice published in the FEDERAL REGISTER or a letter issued under section 512(e) of the act establishing the time within which the requirements of this subpart shall be satisfied.

(1) Notices already published in the FEDERAL REGISTER and letters already sent by the Food and Drug Administration requiring additional studies or submission of an improved regulatory assay will remain in effect, and this subpart will be used in determining compliance with the requirements of the act identified in those notices and letters.

(2) The Commissioner will proceed to withdraw approval of any compound on the basis of data or information indicating a health hazard or in response to any failure to undertake studies necessary to comply with this subpart.

PART 514—NEW ANIMAL DRUG APPLICATIONS

3. In Part 514:

a. By amending § 514.1, by revising paragraph (b)(7) to read as follows:

§ 514.1 Applications.

* * * * *

(b) * * *

(7) *Assays for residues.* A description of practicable methods for determining the quantity, if any, of the new animal drug in or on food, and any substance formed in or on food because of its use, and the proposed tolerance or withdrawal period or other use restrictions for this drug if any tolerance or withdrawal period or other use restrictions are required to ensure that the proposed use of this drug will be safe.

(i) The required information may include: Complete experimental protocols for determining drug residue levels in the edible products, and the time required for residues to be eliminated from the edible products following the drug's use; residue studies conducted under appropriate (i. e., consistent with the proposed usage) conditions of dosage, time, and route of administration to show levels, if any, of the drug and/or its metabolites in test animals during and upon ceasing treatment and at intervals thereafter to establish a depletion curve; if the drug is to be used in combination with other drugs, possible effects of interaction demonstrated by the appropriate disappearance curve or depletion patterns after drug withdrawal under appropriate (i. e., consistent with the proposed usage) conditions of dosage, time, and route of administration; if the drug is given in the feed or water, appropriate consumption records of the medicated feed or water and appropriate performance data in the treated animal; if the drug is to be used in more than one species, drug residue studies or appropriate metabolic studies conducted for each food-producing species. Appropriate use of labeled compounds (e.g., radioactive tracers) may be used to establish metabolism and depletion curves. Drug residue levels ordinarily should be determined in muscle, liver, kidney, fat, and where applicable, in skin, milk, and eggs (yolk and white). As a part of the metabolic studies, levels of the drug or metabolite should be determined in blood when feasible. Samples may be combined if necessary. When residues are suspected or known to be present in litter from treated animals, it may be necessary to include data on those residues' becoming components of other agricultural commodities because of the use of litter from treated animals.

(ii) If the new animal drug has the potential to contaminate human food with residues (parent compound, metabolites, conversion products, or other substances found in or on food because of the drug's use) whose consumption could engender a human risk of carcinogenicity, the applicant and the new animal drug are subject to the requirements of Subpart E o. Part 500 of this chapter.

* * * * *

b. By amending § 514.111, by adding a new paragraph (a)(10) to read as follows:

§ 514.111 Refusal to approve an application.

(a) * * *

(10) The drug fails to satisfy the requirements of Subpart E of Part 500 of this chapter.

* * * * *

PART 571—FOOD ADDITIVE PETITIONS

4. In Part 571, by adding new § 571.115, to read as follows:

§ 571.115 Application of the anticancer clause of section 409.

Food additives intended for use as an ingredient in food for animals that are raised for food production must satisfy the requirements of Subpart E of Part 500 of this chapter.

Interested persons may, on or before May 21, 1979, submit to the hearing Clerk (HFA-305), Food and Drug Administration, Rm. 4-65, 5600 fishers Lane, Rockville, MD 20857, written comments regarding this proposal. Four copies of all comments shall be submitted, except that individuals may submit single copies of comments, and shall be identified with the hearing Clerk docket number found in brackets in the heading of this document. Received comments may be seen in the above office between the hours of 9 a.m. and 4 p.m., Monday through Friday.

In accordance with Executive Order 12044, the economic effects of this proposal have been carefully analyzed, and it has been determined that the proposed rulemaking does not involve major economic consequences as defined by that order. A copy of the regulatory analysis assessment supporting this determination is on file with the Hearing Clerk, Food and Drug Administration.

Dated: February 26, 1979.

SHERWIN GARDNER,
Acting Commissioner of Food and Drugs.

NOTE.—Incorporations by reference provisions approved by the Director of the Office of the Federal Register on December 21, 1978 and on file in the library of that office.

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